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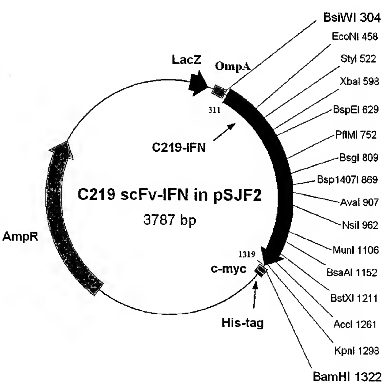
(54) Title: CYTOKINE RECEPTOR BINDING PEPTIDES



Panel D) Plasmid map of the cIFNscFv construct in pSJF2 vector. The construct was directionally cloned from BsiWI to BamHI. The unique restriction sites introduced into the construct are indicated. Numbers beside the restriction sites refer to nucleotide numbers within the construct.

(57) Abstract: The invention relates to cytokine receptor binding peptide constructs and methods of producing same. Preferably the constructs are interferon receptor binding peptide constructs comprising at least one interferon, receptor binding domain inserted into an appropriate scaffold that maintains the binding domain in a configuration suitable for binding to the interferon, receptor. Preferably, the interferon binding peptide constructs comprise three interferon binding domains, one from each of the interferon binding domains of human interferon type 1 (amino acid regions 10-35, 78-107 and 123- 166, preferably 29-35, 78-95 and 130-140, respectively), the scaffold maintaining the three domains in a configuration suitable for binding to their binding sites on the interferon type I receptor. The resulting construct preferably has a biological activity comparable to naturally occurring interferon.

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Title: Cytokine Receptor Binding Peptides

Prior Application

This application claims priority from United States Patent Application No. 09/727,388 filed December 1, 2000, entitled "Cytokine Receptor Binding Peptides", the entirety of which is incorporated by reference herein.

Field of the Invention

The invention relates generally to cytokine receptor binding peptide constructs comprising at least one cytokine receptor binding domain incorporated into a molecular scaffold that maintains the binding domain in a configuration suitable for binding the cytokine receptor and to nucleic acid sequences encoding the same. More particularly, the invention relates to interferon receptor binding peptide constructs. In another embodiment, the invention relates to the general field of cytokine mimetics and more particularly to an interferon mimetic.

Background of the Invention

Cytokines are small, secreted proteins, produced by cells in response to different stimuli. They exert widespread effects on the immune system and target tissues and organs. Cytokines are usually not stored, but quickly made and released in response to stimuli. They bind to their specific cognate receptors. Cytokines are essential for immunoregulation, both positive amplification of the immune response and negative immunosuppression to keep the response from getting out of control.

Accumulating evidence indicates that cytokines, regulate the proliferation and differentiation of target cells, exhibit anti-viral and anti-tumor activities and immune modulating properties. Currently, cytokines are in clinical use in chemotherapies against solid tumors and hematological malignancies and viral infections [1-9]. Moreover, the implications are that cytokines or cytokine antagonists have therapeutic potential in autoimmune diseases, e.g. interferon (IFN-beta) in multiple sclerosis (M.S.) [10]. Despite these indications, only limited success has been achieved with cytokine therapies. The wide variety of dose-limiting and life threatening adverse reactions to systemic cytokine administration, such as metabolic, hemodynamic, renal and cutaneous abnormalities or toxicities are routinely associated with intravenous and subcutaneous administration of cytokines, greatly limits the current effectiveness

of cytokine therapy. There is a need for peptides which mimic cytokine (cytokine mimetics) activities but that have reduced associated side effects.

A class of cytokines, known as interferons (hereinafter referred to as IFNs) are a family of biologically active proteins. IFNs affect a wide variety of cellular functions, related to cell growth control, the regulation of immune responses and the induction of antiviral responses. The ability of IFNs to modulate cell growth is observed with many cell types and is particularly effective in the case of tumor cells, which has led to the widespread interest in the use of IFNs for the treatment of neoplasias.

Interferons (IFNs) were first identified as biologically active molecules capable of inhibiting viral replication in cultured cells [11]. Subsequent purification and cloning of IFNs led to their classification into two distinct groups: Type I IFNs include IFN-alpha, -beta and –omega (IFN-α, -β, and - σ) and Type II IFN or IFN-gamma (IFN-γ) [reviewed in 12]. The Type I IFNs mediate diverse biological effects including the largely cell type-independent antiviral and anti-proliferative responses, and several cell type-restricted responses of immunological relevance such as the activation of natural killer cells, the regulation of CD8+ memory T-cells and the stimulation of hematopoietic cell differentiation [13-16]. These phenotypic changes are thought to be the cumulative consequence of changes in the activity of certain proteins that are products of Type I IFN-stimulated genes (ISGs) [17]. As a result, much effort has been focused on trying to understand the mechanism of Type I IFN signal transduction and regulation of gene expression.

The presence of a specific receptor at the cell surface is the first requirement for IFN action. Cells that lack these specific receptors are resistant to the effects of IFN. Receptor binding studies have identified the existence of at least two functional IFN receptors that are integral parts of the cell membrane on human cells. Branca, A.A. and Baglioni, C., (1981) *Nature* 294, 768-770 [18] report that IFN-alpha and IFN-beta bind to one type of receptor and Anderson, P. et al, (1982) *J. Biol. Chem.* 257, 11301-11304 [19] report that IFN-gamma binds to a separate receptor. IFN receptors are ubiquitous and more specifically, are up-regulated in metabolically active cells such as cancer cells and infected tissues. Although several of the effects of IFNs such as the antiviral state, take several hours to develop, signal transduction

immediately following the binding of IFN to its receptor is a rapid event. Since metabolic changes, such as genes can be detected within five minutes of the addition of IFN, at least some of the transmembrane signals must be very rapid. Hannigan et al, (1986) *EMBO J.* 5, 1607-1613 [20] suggest that receptor occupancy modulates the transcriptional response of specific genes to IFN. Indeed, there is accumulating evidence to suggest that there is a direct relationship between the number of receptors occupied and the amount of signal that is transduced to the cell nucleus. These transduced signals result in altered gene expression in the nucleus, which mediates the subsequent biological responses.

Extensive studies were undertaken to define those critical clusters of amino acids in the different IFN-alphas and IFN-beta that interact with the Type I IFN receptor complex. It is thought that these critical peptide domains would serve as prototypes for synthetic peptides that are useful as carriers for pharmaceutical compositions. The present inventor has identified three IFN Type I receptor binding domains in human Type I IFNs (US Patent No. 5,684,129, issued November 4, 1997) that effect responses in target cells: amino acid residues 10-35 (interferon receptor recognition site I), 78-107 (interferon receptor recognition site 2) and 123-166 (interferon recognition site III). The present inventor also determined that peptides comprising portions of these domains also possess biological activity (ability to bind to the IFN receptor) such as those comprising amino acid residues 29-35, 78-95, 123-140 or 130-140. Further the present inventor identified seven preferred interferon receptor binding peptides which have an amino acid sequence selected from the group consisting of: CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1); ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2); ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3); TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); and GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7).

It is not unusual for cytokines to have more than one receptor interactive domain. All cytokines exert their functional effects through cell surface expressed

receptor complexes. These are comprised of transmembrane subunit components. Generally, cytokine receptor systems associated with helix bundle cytokines consist of at least 2 types of subunits. For a number of different cytokines, separate sites for recognition of the different receptor subunits have been mapped in the cytokines. For IL-5 [21], GM-CSF [22], and growth hormone [23], the presence of distinct receptor interaction sites for the different receptor subunits is consistent with a sequential receptor-subunit-binding scheme. The cytokine initially binds with low affinity to one receptor subunit, followed by the interaction with the second subunit, thereby creating a high affinity receptor complex that mediates signal transduction.

Structure of Cytokines

Structurally, many cytokines, including IFNs are constructed from a common helix bundle structural framework. Rapid advances have been made in relating the structure and function of a growing number of four-helix bundle cytokines. For example, IL-5 and GM-CSF are closely related four-helix bundle growth factor proteins. GM-CSF has a core structure composed of a left-handed, antiparallel four-helix bundle with up-up-down-down connectivity similar to the fold first described for growth hormone [24]. IL-5 comprises a core of two four-helix bundles, each of which is composed of a D helix from one polypeptide chain of the IL-5 dimer and three helices from the second chain [25]. Yet, even as a two bundle system, the helix bundle motifs in IL-5 are similar to those of growth hormone, GM-CSF and, indeed, to those of many other cytokines including IL-2, IL-3, IL-4, M-CSF and G-CSF. A similar fold can be predicted for other cytokines, including Epo, IL-6, IL-7, IL-10, IL-12 and prolactin, based on gene structure and homology modelling. Beyond the common helical core, all known four-helix bundle cytokines contain common ß-sheet elements formed by the strand connecting the A and B helices and the strand connecting helices C and D [reviewed in 26]. The helical bundle structure of IFN conforms with other cytokines.

Structure of Immunoglobulins

Immunoglobulins in general are comprised of two polypeptide chains of different lengths, the light (L) and heavy (H) chains. Two of each of these chains associate to form one antibody molecule. The functions of an antibody can be assigned to structural domains, termed the Fab (antigen-binding fragment) and Fc

(constant or crystallizable fragment) [27]. The Fab is responsible for molecule-specific functions, such as antigen recognition, while the Fc carries out the activities of antibodies in general, such as complement activation and receptor binding.

The crystal structures of a number of Fabs revealed a characteristic protein folding pattern which consisted of two anti-parallel-sheets tied together by an internal disulphide bond [28]. Two of these 'immunoglobulin fold' units associate together non-covalently to form a double-cylindrical barrel structure. The hypervariable stretches described above occur in inter-strand loops, all at one end of the barrel. Thus, although distant in the primary sequences of the H and L chains, all six of these stretches come together at one end of the antibody to form a single binding surface. As the presence of haptens in the structures identified this surface as the antigen-binding site, the stretches became known as complementarity-determining regions (CDRs). General conclusions have been drawn regarding antibody recognition, such as the predominance of hydrophobic interactions, mediated largely by aromatic Tyr, Phe and Trp residues, with a smaller number of specific polar contacts between antibody residues and substituents on the antigens. Perhaps as revealing has been the different shapes of the binding regions. Interactions between antibodies and large proteins usually involve the association between relatively flat faces, while smaller molecules tend to be buried in pockets within the antibody site. Carbohydrates can either lie in crevices, in the example of long-chain polysaccharides, or in pockets, where the end of the sugar chain is recognized. The shape of the antigen-binding site is mostly governed by the length and nature of the six CDRs. In general, the first two CDRs of each of the heavy and light chains (H1, H2, L1, L2) form the periphery of the site, establishing the general features of the shape. The details, and usually most of the specificity determining interactions, are provided by the third CDRs L3 and, especially, H3). These loops usually form the center of the binding site. Shorter H3 and L3 loops tend to fold down, creating shallow or flat sites, while longer loops usually result in deeper, smaller pockets.

The application of recombinant DNA technologies to molecules based on the antibody structure has led to the field of antibody engineering [reviewed in 29]. For most purposes, only the antigen-binding portion of the antibody needs to be expressed. The most common approach to this has been to take advantage of the

intrinsic association properties of the immunoglobulin variable regions, and to express the genes encoding the V portions of the heavy and light chains (Vh and Vl) alone. When co-expressed, these units will normally self-associate to form the variable domain (Fv) containing the entire antigen-binding site, though this domain cannot normally be produced by enzymatic cleavage of intact antibodies. In order to facilitate the co-expression, the two gene fragments are usually linked together into a single chain product (scFv) with a segment encoding a flexible linker peptide region.

The primary advantages of this approach has been the relatively small size of the specific binding molecule compared to an intact antibody and, secondly, the potential to over express large amounts of protein product without the use of animals. The antibody domain genes can be expressed fused to the genes encoding surface proteins of bacteriophage. If a large library of such engineered phage is assembled, an Fv of desired specificity can be isolated in most instances by a selection process. The affinity can be improved further by an affinity maturation process [30]. Libraries of such phage with a large number of different Fv fusions are now available and have been used successfully in antibody evolution experiments. Thus, the production of large amounts of high affinity Fv products with a desired specificity is possible. Indeed, with regard to the scFv described below, this recombinant product may be expressed in relatively large amounts and reproducibly folds appropriately [31].

Summary of the Invention

The inventors have determined that the presence of multiple interferon binding domains in the interferon molecule and, the conservation of the highly structured helical bundle configuration amongst the cytokines, including IFNs, that potentially represents a stable structure that 'presents' the receptor interactive domains of the different cytokines, enable their interaction with the corresponding receptor contact site(s). The present inventors have determined that the configuration of the interferon receptor binding domains, or critical parts thereof, in the native IFN and the subsequent interaction with the IFN receptor, is important in effecting biological outcomes or responses and thus is important to the design of an IFN mimetic. Further, the present inventors have determined that the ability of the recombinant scFv molecules to be reproducibly made in relatively large amounts in a predictable

configuration, make them excellent candidates for scaffolds onto which peptide interactive domains of cytokines may be inserted.

In one embodiment, the present invention provides a cytokine receptor binding peptide construct comprising at least one cytokine receptor binding domain incorporated in a suitable molecular scaffold such that the scaffold maintains the binding domain in a configuration suitable for binding to the cytokine receptor.

For the purposes of the present invention, the term "scaffold" is defined as any structure that can be manipulated for the purpose of optimizing the spatial positioning of inserted peptides. Suitable scaffolds for use in the present invention may include, but are not limited to polypeptides, proteins, carbohydrates, glycoproteins, synthetic and natural polymers, antibody or any other molecule or substance that may be apparent to one skilled in the art. Preferably the scaffold is a scFv molecule, most preferably a C219scFv molecule.

Further, the present inventors have now found that the binding domains of cytokines can be incorporated into scFv peptide molecules (as the molecular scaffold) in a stable and reproducible configuration. These smaller hybrid molecules that contain the effective portions of the cytokine (i.e. cytokine receptor binding domains) can be used to mimic the effects of the relatively large native cytokine, with preferably reduced side effects.

Thus in one embodiment, the present invention provides a novel cytokine receptor binding peptide construct comprising at least one cytokine receptor binding domain incorporated into an scFv peptide molecule in a manner that maintains the binding domain in a configuration suitable for binding the cytokine receptor.

In one embodiment of the invention, the cytokine peptide comprising the cytokine receptor binding domain is incorporated into the scFv peptide at one of the complimentarity determining regions (CDRs, i.e., CDR1, CDR2 or CDR3).

In another embodiment, the cytokine is interferon, preferably a Type I human interferon. Thus in a further aspect, the present invention provides the insertion of one or more interferon peptides comprising an interferon receptor binding domain into strategic locations along a scaffold, facilitating interaction of one or more of said peptides with the corresponding active site of the Type 1 human interferon receptor.

Preferably, one of the said interferon peptides is inserted at one of the CDR sites of scFv, thereby altering the respective CDR of scFv.

In another embodiment of the invention, the interferon peptides incorporated into the suitable scaffold comprise at least an interferon receptor binding domain selected from the group of IFN peptides comprising the IFN amino acid residues: 10-35 (interferon receptor recognition site I), 78-107 (interferon receptor recognition site 2) and 123- 166 (interferon recognition site III) or IFN receptor binding portions thereof. Most preferably the interferon peptides incorporated into the scaffold of the invention comprise at least one of the following interferon amino acid residue domains: amino acid residues 29-35 (IFN receptor recognition peptide I (IRRP1)), 78-95 (IFN receptor recognition peptide II (IRRP2)), a 79-96 or 89-95 (both from IFN recognition site II), 123-140 (interferon receptor recognition peptide III (IRRP3) 130-140 (from IFN recognition site III), 123-156 (from IFN recognition site III) or 123-129 or 129-140 (from IFN recognition site III). Most preferably, the interferon receptor binding peptides incorporated into the scaffold comprise the amino acid sequence selected from the group consisting of: CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1); ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2); ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3); TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7), GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP-LEU(SEQ.ID.NO.20), and LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA(SEQ. ID. NO. 21).

In another embodiment, the interferon receptor binding peptide construct of the invention comprises at least 2 and preferably 3 IFN receptor binding domains, one from each of the three IFN receptor recognition sites or IFN receptor binding portions thereof and each replacing a different CDR in the scFv scaffold preferably in a configuration which enables the IFN receptor binding domains to interact with their corresponding active sites in the interferon receptor. In one embodiment the IFN

receptor recognition sites or binding portions thereof are inserted in the same chain of the scFv scaffold, in another embodiment, they are not all inserted on the same chain.

In one embodiment, a Type I human IFN receptor binding peptide of domain 1 is incorporated at CDR3 and alters CDR3, Type I human IFN receptor binding peptide of domain 2 is incorporated at CDR2 and alters CDR2, and Type II human IFN receptor binding peptide of domain 3 is incorporated at CDR1 and alters CDR1. In a further embodiment, only one or two IFN receptor recognition sites or binding portions thereof are incorporated into the scaffold.

In a preferred embodiment, the invention provides an IFN receptor binding peptide construct comprising the amino acid sequence of SEQ. ID No. 22 (pIFNscFv - C219 scaffold with inserted interferon peptides SEQ. ID. NO. 1 at CDR1 of the variable light chain of scFv and SEQ. ID. NO. 21 at CDR3 of variable light chain of scFv. In another embodiment the construct has the amino acid sequence encoded by SEQ.ID.NO.29 or a different nucleic acid sequence coding for the same amino acid sequence due to the degeneracy of the genetic code.

In another embodiment, a Type I human IFN receptor binding peptide of domain 1 is incorporated at CDR1, preferably the VH chain of scFv, and alters CDR1; Type I human IFN receptor binding peptide of domain 2 is incorporated at CDR2 and alters CDR2, preferably in the VL chain of scFv, and Type I human IFN receptor binding peptide of domain 3 is incorporated at CDR3 and alters CDR3, preferably in the VH chain of scFv. In a further embodiment, three IFN receptor recognition sites or binding portions thereof are incorporated into the scaffold.

In another embodiment, the invention provides an IFN receptor binding peptide construct encoded by SEQ. ID. NO. 32 or a different nucleotide sequence encoding for the same amino acid sequence construct as that encoded for by SEQ. ID. NO. 32 due to the degeneracy of the genetic code. In another embodiment, the IFN receptor binding peptide construct has the amino acid sequence of SEQ. ID. NO. 33.

In another aspect the invention provides for nucleic acid molecules encoding the cytokine, preferably IFN, receptor binding peptides of the invention. In another embodiment the invention provides suitable expression vectors which incorporate the nucleic acid molecules of the invention. Preferably the expression vector is plasmid, pSJF2. Most preferably the invention provides a nucleic acid sequence encoding an

interferon receptor binding peptide construct of the invention and preferably comprising the nucleic acid sequence of SEQ. ID. NO.29 or SEQ. ID. NO. 32. An embodiment of this aspect of the invention provides the protein expressed from the plasmid of the nucleic acid sequence provided in SEQ. ID NO. 29 or SEQ. ID. NO. 32.

In yet another embodiment, the invention provides for a cell genetically modified, either through transformation, transfection or other means to express the IFNscFv , preferably a partial (less than three IFN receptor recognition sites or receptor binding portions thereof) or complete (three IFN receptor recognition sites or binding protions thereof) IfnscFv, protein of the invention.

In one embodiment, the invention provides for a pharmaceutical composition comprising one of the peptides of the invention and a pharmaceutically acceptable carrier. Preferably, the pharmaceutical composition comprises an interferon receptor binding peptide construct of the invention and a pharmaceutically acceptable carrier

In a further embodiment the invention provides a peptide that mimics the effect of IFN and which can be used in medical therapies for cancers, hematological malignancies, viral infections and autoimmune diseases.

In a further embodiment, the invention provides a peptide that can be used to detect modulators of IFN action. For instance the IFN mimetics of the invention can be used in screening assays to compare the activity and/or interaction with another molecule or potential IFN modulator. This can be done by observing the effect of the modulator in the presence of the peptide mimetic of the invention and its substrate (e.g. the IFN receptor). The peptides of the invention can also be used in the diagnosis of IFN activity related disorders, by comparing the effect of the peptide or modulators of the peptide to the invention on IFN activity in a person with the disorder. This can be done by taking a sample from the person with the disorder, such as a blood tissue sample and observing the effect of peptide mimetic subtrate interaction in the presence of the sample and comparing with that of an individual with a known IFN disorder state (normal, abnormal or both).

In yet another embodiment, other factors can be attached to, operatively linked to or incorporated into the scaffold. As such the interferon receptor binding peptides and peptide constructs of the invention can be used as a carrier for other therapeutie

agents. For instance, another therapeutic agent could be attached to the scaffold, to provide a bolus of, for example, interferon and another chemotherapeutic agent, or IFN and ribavirin, or IFN and an anti-inflammatory agent.

In another embodiment the invention provides a method of producing a cytokine mimetic comprising:

(i) comparing the configuration of a cytokine receptor binding domain with that

of an scFv molecule;

(ii) determining a suitable site for insertion of the binding domain in the scFv molecule to ensure maintenance of the domains in a configuration suitable for binding the cytokine receptor;

(iii) inserting the binding domain in the said identified site from step (ii) in a manner which enables the binding domain to bind the corresponding site of the receptor, altering the native scFv molecules; optionally

(iv) repeating steps (i) to (iii) for each cytokine receptor binding domain for cytokines that have more than one domain.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Brief Description of the Drawings

FIG. 1 illustrates the growth inhibitory activities of variant IFN-alphas in T98G cells.

FIG. 2 shows five charts illustrating receptor binding characteristics of variant IFN-alphas on T98G cells.

FIG. 3 shows four charts illustrating receptor binding characteristics of variant IFN-alphas on T98G cells.

FIG. 4 shows secondary structure characteristics of different IFN-alpha species according to amino acid sequence analyses.

FIG. 5 is a representation of a model for the tertiary structure of Type 1 IFNs.

FIG. 6A is a Ni column purification of IFNscfv from concentrated periplasmic extract dialysed against 10mM Tris pH 8 buffer containing 100 mM NaCl.

FIG. 6B is a Western blot showing the size of the IFNscfv

FIG. 7 is a silver stain of Q-column purified protein from Ni column pooled fractions dialysed against 50mM Tris pH 8.

FIG. 8A is a consensus interferon alpha carbon trace, wherein the interferon receptor binding regions are highlighted.

FIG 8B is a consensus C219scFv carbon trace, wherein the CDRs in variable light chain regions are highlighted.

FIG. 9A is a bar graph illustrating the effect of pIFNscFv on Daudi cell growth. The data semonstrate that addition of C219scFv alone to Daudi cells does not affect their growth. However, addition of the partial mimetic (pIFNscFv) appears to inhibit the growth-regulatory effects of endogenously produced IFN in Daudi cells. FIG. 9 B is a bar groaph illustrating that pIFNscFv can partially rescue Daudi cells from the growth inhibitory effects of IFN-Con. The data demonstrate that at high doses of IFN, that exhibit maximal growth inhibitory activity, competition with the partial mimetic results in some ablation of the IFN-induced growth inhibitory effects at the highest doses of competitor mimetic.

FIG. 10 shows the nucleotide sequence that were removed from the C219scFv cDNA and IFN nucleotide sequences that were placed in the CDRs.

FIG.11 Plasmid map for the cIFNscFv construct in pSJF2 vector. The construct was directionally cloned from BsiWI to BanHI. The unique restriction sites introduced into the construct are indicated. Numbers beside the restriction sites refer to nucleotide numbers within the construct.

FIG.12 Anti-c-Myc Western immunoblot (12% SDS-PAGE) of whole cell lysates to screen for presence of the c-Myc tagges cIFNscFv mimetic. An IPTG inducible band, which corresponds to the expected molecular weight of the cIFNscFv mimetic, is present in lane 4. The produce is about 37 kDa. Two bands are seen on the immunoblot. The higher band corresponds to unprocessed protein i.e., signal sequence is still present.

FIG.13 Nucleic acid and amino acid sequence of cIFNscFv

FIG. 14 IFN-α primary amino acid sequences and sequence alignment.

FIG. 15 Nucleic and amino acid sequence of C219scFv. SEQ. ID. NO 18 is from nucleic acid sequence 76 – 831). SEQ. ID. NO. 19 is from amino acid sequence 26-277.

Detailed Description of Invention

The present invention is directed to cytokine receptor binding peptide constructs, wherein the receptor binding domain of the cytokine is incorporated into a scaffold such that the scaffold maintains the binding domain in a configuration suitable for binding to the cytokine receptor. The scaffold is preferably an inert (preferably immunologically inert) biological scaffold that preferably optimizes the spatial positioning of inserted peptides. Suitable scaffolds for use in the present invention may include, but are not limited to polypeptides, proteins, carbohydrates, glycoproteins, synthetic and natural polymers, or any other molecule or substence that may be apparent to one skilled in the art. Preferably the scaffold is an immunoglobulin or fragment thereof. Most preferably the scaffold comprises the variable antigen binding domain of an immunoglobulin. Most preferably the scaffold is a single chain Fv peptide (scFv), such as C219scFv. The scaffold is preferably easily and predictably reproducible, with a predictable spatial configuration.

Biologically active proteins have an optimum active configuration that is composed of discrete and unique strategic domains along the polypeptide. These critical structural domains determine such parameters as receptor binding and effector functions. Characterization of these strategic domains, that includes defining their spatial configuration and effector functions, clarify the sequence of events comprising and initiated by receptor binding and that lead to specific biological responses. Defining the spatial configuration of the strategic domains can help in designing peptides which may mimic all or some of the functions of the biologically active proteins.

A receptor may be either activated by a ligand/agonist or inhibited/desensitized by a receptor antagonist, resulting in a variety of biological effects. The peptides of the present invention are incorporated into a biological scaffold. Their position within the scaffold will determine the degree of interaction with the receptor active sites, resulting in various degrees of stimulation.

For a therapeutic agent to be optimally active, it must be delivered to the specific site of action intact and must interact with the target tissues. In a number of clinical conditions, such as uncontrolled proliferation in neoplastic tissues, or infected tissues, or inflamed tissues, the cells express abundant Type I IFN receptors, that is, IFN-alpha/beta receptor expression at the cell surface is upregulated. It has been determined that specific peptides are capable of recognizing and binding to these cell surface receptors. Once bound, the ligand-IFN receptor complex is internalized into the cell.

The Peptides of the Invention

The following standard abbreviations for the amino acid residues are used throughout the specification: A, Ala - alanine; C, Cys - cysteine; D, Asp- aspartic acid; E, Glu - glutamic acid; F, Phe - phenylalanine; G, Gly - glycine; H, His - histidine; I, Ile - isoleucine; K, Lys - lysine; L, Leu - leucine; M, Met - methionine; N, Asn - asparagine; P, Pro - proline; Q, Gln - glutamine; R, Arg - arginine; S, Ser - serine; T, Thr - threonine; V, Val - valine; W, Trp- tryptophan; Y, Tyr - tyrosine; and p.Y., P.Tyr - phosphotyrosine.

Also, reference to IFN amino acid residue numbers conform to the numbering of the residues in Table 1 and Figure 14 herein.

The peptide sequence of a number of human Type I interferons is shown in Table I and Figure 14. The present inventor previously identified three human Type I interferon receptor binding domains, from amino acid residues 10-35 (domain 1), 78-107 (domain 2) and 123-166 (domain 3) and more particularly from amino acid residues 29-35, 78-95 and 123-140, or 130-140 or 123-156. Peptides comprising an amino acid sequence of anyone of these regions or biologically active fragments thereof, in a suitable configuration for binding to the receptor are encompassed within the scope of the present invention. Peptides comprising an amino acid sequence of anyone of these regions includes peptides which may comprise additional amino acid residues at the flanking regions of the sequences, but that do not inhibit the binding activity of the receptor binding domains. The peptides per se that are incorporated into the scaffold of the invention are also encompassed within the scope of the present invention.

"Cytokine receptor binding domain" as used herein refers to any peptide that can bind to the corresponding cytokine receptor but may include other regions or additional amino acid residues that do not inhibit the binding activity of the peptide. "Interferon receptor binding domain" shall have a corresponding meaning.

The term "biologically active fragment thereof" as used herein means any fragment that can bind the respective cytokine receptor.

Thus the present invention provides a novel cytokine receptor binding peptide construct comprising at least one cytokine receptor binding domain incorporated into an scFv peptide molecule, such as scFv, preferably having the amino acid sequence of SEQ. ID. NO. 18 (or Figure 15) in a manner that maintains the binding domain in a configuration suitable for binding the cytokine receptor.

In one embodiment of the invention, the cytokine peptide comprising the cytokine receptor binding domain is incorporated into the scFv peptide at one of the complimentarity determining regions (CDRs, i.e., CDR1, CDR2 or CDR3) although other sites of incorporation may also be suitable. The scFv peptide would be altered at the site of incorporation. The cytokine binding domain can be incorporated into the scFv peptide by replacing all or part of a region of scFv (e.g. all or part of CDR1, CDR2 and/or CDR3) or by adding the sequence to the scFv at a particular site.

In another embodiment, the cytokine is interferon, preferably a Type I human interferon. Thus in a further aspect, the present invention provides the insertion of one or more interferon peptides which bind the IFN receptor into strategic locations along a scaffold, facilitating interaction of one or more of said peptides with the corresponding active site of the Type 1 human interferon receptor. Preferably, one of the said interferon peptides is inserted at one of the CDRs of scFv, altering the respective CDR of scFv.

In another embodiment of the invention, the interferon peptides incorporated into the suitable scaffold comprise at least an interferon receptor binding domain selected from IFN amino acid residues: 10-35 (interferon receptor recognition site I), 78-107 (interferon receptor recognition site 2) and 123- 166 (interferon recognition site III) or IFN receptor binding portions thereof. Most preferably the interferon peptides incorporated into the scaffold of the invention comprise at least one of the following amino acid residue domains: amino acid residues 29-35 (IRRP1), 78-95

(IRRP2) (or in other embodiments 89-95 or 79-96) and 123-140 (IRRP3) (or in other embodiments 130-140 or 123-156). Most preferably, the interferon receptor binding peptides incorporated into the scaffold comprise the amino acid sequence selected from the group consisting of: CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1); ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2); ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3); TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7), GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP-LEU(SEQ.ID.NO. 20), and LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA(SEQ. ID. NO. 21).

In another embodiment, the interferon receptor binding peptide construct of the invention comprises at least 2 and preferably 3 IFN receptor binding domains, one from each of the three IFN receptor recognition sites or respective IFN receptor binding portions thereof and each replacing a different CDR in the scFv scaffold.

It would be appreciated that the many configurations can be constructed. Although specific constructs have been made, the invention is not intended to be limited to such constructs. Optimum configurations (for instance IRRP1, IRRP2, or IRRP3 could be inserted at anyone of the CDR regions depending on whether a partial mimetic or construct is being made (ie., one with one or two IFN receptor binding peptide insertions, such as SEQ. ID. NO. 22, pIFNscFv) or whether a full mimetic, (i.e. with an IRRP inserted at each of the CDR sites, such as SEQ. ID. NO. 33 or Figure 13, cIFNscFV). Optimum insertion sites and structures may change depending on factors such as steric hinderance. The molecular structures of the cytokines, such IFNs , and scaffold, such as scFv can be obtained by conventional techniques in the art. Known structures can be obtained from various databases, such the Protein Data Bank at http://www.rscb.org/pdb.

The protein structures of the scaffold and the cytokine, such as IFN, and respective IFN receptor binding domains or amino acid sequences comprising such domains or IFN receptor binding portions thereof, can be used determine optimum insertion sites to obtain IFN receptor binding using known molecular modeling factors, such as bond lengths or bond stress. The IFN receptor binding domains or portions thereof and their respective nucleic acid coding sequences can be incorporated in various permutations into the CDR regions of the scaffold or coding regions therefore. Optimum configurations can be predicted using techniques known in the art, such as computer modelling and confirmed by subsequent construction, expression and activity assays.

In one embodiment, Type I human IFN receptor binding peptide of domain 1 (comprising amino acid residues of IFN receptor recognition site I or IFN receptor binding portion thereof), is incorporated at CDR1, preferably at VH of svFv and alters CDR1, Type I human IFN receptor binding peptide of domain 2 (comprising amino acid residues of IFN receptor recognition site II or IFN receptor binding portion thereof), is incorporated at CDR2 peptide of domain 2 (comprising amino acid residues of IFN receptor recognition site II or IFN receptor binding portion thereof), is incorporated at CDR2 and alters CDR2; and/or Type I human IFN receptor binding peptide of domain 3 (comprising amino acid residues of IFN receptor recognition site III or IFN receptor binding portion thereof), is incorporated at CDR1, preferably of VL of scFv and alters CDR1. This was issued in construct of SEQ. ID. NO. 22 when a pIFNscFv was constructed. However, preferably of VL of scFv and alters CDR2; and/or Type I human IFN receptor binding peptide of domain 3 (comprising amino acid residues of IFN receptor recognition site III or IFN receptor binding portion thereof), is incorporated at CDR3, preferably of VL of scFv, and alters CDR3. This was used in construct of SEQ.ID No.33 (Figure 13) for the cIFNscFv was constructed. However, other suitable configurations are possible.

In another embodiment, Type I human IFN receptor binding peptide of domain 1 (comprising amino acid residues of IFN receptor recognition site I or IFN receptor binding portion thereof), is incorporated at CDR3, preferably of VL of scFv and alters CDR3, Type I human IFN receptor binding other suitable configurations are possible.

In a preferred embodiment, the invention provides an IFN receptor binding peptide construct comprising the amino acid sequence of SEQ.ID No.22 (pIFNscFv - C219 scaffold with inserted interferon peptides) or SEQ.ID No.33 (cIFNscFv-219 scaffold with inserted interteron peptides). In another embodiment the invention provides a peptide encoded by the nucleic acid sequence of SEQ. ID. NO. 29 or 32 (Figure 13) or a degenerate sequence encoding the same amino acid molecule.

In yet another embodiment, other factors can be attached to, operatively linked to or incorporated into the scaffold. As such the interferon receptor binding peptides and constructs of the invention can be used as a carrier for other therapeutic agents. For instance, another therapeutic agent could be attached to the scaffold, to provide a bolus of, for example, interferon and another chemotherapeutic agent, or IFN and an ribavirin, or IFN and anti-inflammatory agent.

In addition to the full length amino acid sequences of the invention (e.g. SEQ. ID. No. 22 of 33) the proteins of the present invention may also include truncations of the proteins, and analogs, and homologs of the proteins and truncations thereof as described herein. The truncated proteins included in the invention are those that can still bind the respective cytokine receptor.

Analogs of the proteins having the amino acid sequences of the invention, preferably SEQ. ID. NO. 22 or 33 (Figure 13) and/or truncations thereof as described herein, may include, but are not limited to an amino acid sequence containing one or more amino acid substitutions, insertions, and/or deletions. Amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions involve replacing one or more amino acids of the proteins of the invention with amino acids of similar charge, size, and/or hydrophobicity characteristics. When only conserved substitutions are made the resulting analog should be functionally equivalent. Non-conserved substitutions involve replacing one or more amino acids of the amino acid sequence with one or more amino acids which possess dissimilar charge, size, and/or hydrophobicity characteristics.

One or more amino acid insertions may be introduced into the amino acid sequences shown in SEQ. ID. NO 22 or 33 (Figure 13). Amino acid insertions may consist of single amino acid residues or sequential amino acids ranging from 2 to 15 amino acids in length. For example, amino acid insertions may be used to destroy

target sequences so that the protein is no longer active. This procedure may be used in vivo to inhibit or compete with the activity of a protein of the invention or a particular cytokine.

Deletions may consist of the removal of one or more amino acids, or discrete portions from the amino acid sequence shown in SEQ. ID. NO.22 or 33 (Figure 13). The deleted amino acids may or may not be contiguous. The lower limit length of the resulting analog with a deletion mutation is about 10 amino acids, preferably 100 amino acids.

Analogs of the proteins of the invention may be prepared by introducing mutations in the nucleotide sequence encoding the protein. Mutations in nucleotide sequences constructed for expression of analogs of a protein of the invention must preserve the reading frame of the coding sequences. Furthermore, the mutations will preferably not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, which could adversely affect translation of the mRNA.

Mutations may be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site specific mutagenesis procedures may be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Deletion or truncation of a protein of the invention may also be constructed by utilizing convenient restriction endonuclease sites adjacent to the desired deletion. Subsequent to restriction, overhangs may be filled in, and the DNA religated. Exemplary methods of making the alterations set forth above are disclosed by Sambrook et al [32].

The proteins of the invention also include homologs of the amino acid sequence of the invention, especially that of SEQ. ID. NO. 22 or 33 (Figure 13) and/or truncations thereof as described herein. Such homologs are proteins whose amino acid sequences are encoded by nucleic acid sequences that hybridize under stringent hybridization conditions (see discussion of stringent hybridization

conditions herein) with a probe used to obtain a protein of the invention. Homologs of a protein of the invention will have the same regions which are characteristic of the protein.

A homologous protein includes a protein with an amino acid sequence having at least 76%, preferably 80-90% identity with the amino acid sequence as shown in SEQ. ID. NO. 22. The invention also contemplates isoforms of the proteins of the invention. An isoform contains the same number and kinds of amino acids as a protein of the invention, but the isoform has a different molecular structure. The isoforms contemplated by the present invention are those having the same properties as a protein of the invention as described herein.

The present invention also includes a protein of the invention conjugated with a selected protein, or a selectable marker protein (see below) to produce fusion proteins. The proteins of the invention (including truncations, analogs, etc.) may be prepared using recombinant DNA methods. These proteins may be purified and/or isolated to various degrees using techniques known in the art. Accordingly, nucleic acid molecules of the present invention having a sequence that encodes a protein of the invention may be incorporated according to procedures known in the art into an appropriate expression vector which ensures good expression of the protein. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is compatible with the host cell used. The expression "vectors suitable for transformation of a host cell", means that the expression vectors contain a nucleic acid molecule of the invention and regulatory sequences, selected on the basis of the host cells to be used for expression, which are operatively linked to the nucleic acid molecule. "Operatively linked" is intended to mean that the nucleic acid is linked to regulatory sequences in a manner which allows expression of the nucleic acid.

The invention therefore contemplates a recombinant expression vector of the invention containing a nucleic acid molecule of the invention, or a fragment thereof, and the necessary regulatory sequences for the transcription and translation of the inserted protein-sequence. Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, or viral genes (For example, see the

regulatory sequences described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990) [33]. Selection of appropriate regulatory sequences is dependent on the host cell chosen, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. It will also be appreciated that the necessary regulatory sequences may be supplied by the native protein and/or its flanking regions.

The recombinant expression vectors of the invention may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention. Examples of selectable marker genes are genes encoding a protein which confers resistance to certain drugs, such as G418 and hygromycin. Examples of other markers which can be used are: green fluorescent protein (GFP), ß-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. Transcription of the selectable marker gene is monitored by changes in the concentration of the selectable marker protein such as b-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. If the selectable marker gene encodes a protein conferring antibiotic resistance such as neomycin resistance transformant cells can be selected with G418. Cells that have incorporated the selectable marker gene will survive, while the other cells die. This makes it possible to visualize and assay for expression of recombinant expression vectors of the invention and in particular to determine the effect of a mutation on expression and phenotype. It will be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

The recombinant expression vectors may also contain genes that encode a fusion moiety which provides increased expression of the recombinant protein; increased solubility of the recombinant protein; and aid in the purification of a target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target recombinant protein to allow

separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein.

Recombinant expression vectors can be introduced into host cells to produce a transformed host cell. The term "transformed host cell" is intended to include prokaryotic and eukaryotic cells which have been transformed or transfected with a recombinant expression vector of the invention. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (e.g. a vector) into a cell by one of many possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium chloride mediated transformation. Nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co precipitation, DEAE-dextran-mediated transfection, lipofectin, electroporation or microinjection. Suitable methods for transforming and transfecting host cells can be found in Sambrook et al. [32] and other such laboratory textbooks.

Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins of the invention may be expressed in bacterial cells such as E. coli, insect cells (using baculovirus), yeast cells or mammalian cells. Other suitable host cells can be found in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1991) [33].

The proteins of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis [34] or synthesis in homogenous solution [35].

The Nucleic Acid Sequences of the Invention

In another aspect the invention provides for nucleic acid molecules encoding the cytokine, preferably IFN, receptor binding peptide constructs of the invention. In another embodiment the invention provides suitable expression vectors which incorporate the nucleic acid molecules of the invention. Preferably the expression vector is plasmid, pSJF2. Most preferably the invention provides a nucleic acid sequence encoding the interferon receptor binding peptide construct of SEQ. ID. NO.29 or SEQ. ID NO.32. An embodiment of this aspect of the invention provides

the protein expressed from the plasmid of the nucleic acid sequence provided in SEQ. ID NO. 29 or SEQ. ID. NO.33 (Figure 13).

In yet another embodiment, the invention provides for a cell genetically modified, either through transformation, transfection or other means to express the IFNscFv protein of the invention. In one embodiment the invention comprise the nucleic acid sequence of Figure 15 or Figure 11 with the novel restriction sites

The present invention provides an isolated nucleic acid molecule comprising a sequence encoding an interferon receptor binding protein. The term "isolated" refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or other chemicals when chemically synthesized. The term "nucleic acid" is intended to include DNA and RNA and can be either double stranded or single stranded.

In an embodiment of the invention, an isolated nucleic acid molecule is provided having a sequence which encodes an interferon receptor binding protein having an amino acid sequence as shown in SEQ ID NO. 22 or SEQ. ID. NO.33 (Figure 13).

Preferably, the isolated nucleic acid molecule comprises

(a) a nucleic acid sequence as shown in SEQ ID NO. 29 or that of SEQ ID NO. 18 (encoding the scFv scaffold) with at least one nucleic acid sequence encoding for an interferon receptor binding domain (such as SEQ. ID. NOS. 1-7, 20 and 21 or any other nucleic acid sequences from the IFN receptor binding domain that bind IFN such as in the sequences in Table 1 or Figure 14 within the IFN receptor binding domains shown) incorporated therein, wherein T can also be U;

(b) nucleic acid sequences complementary to (a);

(c) nucleic acid sequences which are homologous to (a) or (b);

(d) a nucleic acid molecule differing from any of the nucleic acids of (a) to (c) in codon sequences due to the degeneracy of the genetic code; or

(e) a biologically active fragment of (a) to (d) that will hybridize to (a) to (d) under stringent hybridization conditions.

In another embodiment the invention provides a nucleic acid molecule comprising the coding region of a human Type I interferon receptor binding protein , such as SEQ ID NO. 22 or 33or biologically active fragment thereof.

In all of the sequences referred to above, T can also be U. The invention further encompasses, nucleic acid molecules which are complementary in sequence to nucleic acid molecules of the invention, fragments of the nucleic acid molecules of the invention, preferably at least 15 bases, and more preferably of at least 20 to 30 bases, and which will hybridize to the nucleic acid molecules of the invention under stringent hybridization conditions. Appropriate stringency conditions which promote DNA hybridization are known to those skilled in the art, or may be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6 [36]. For example, the following may be employed: 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C. The stringency may be selected based on the conditions used in the wash step. For example, the salt concentration in the wash step can be selected from a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, such as at about 65°C.

The fragments, among other things, can be used as primers to amplify (using techniques known in the art, such as the polymerase chain reaction) or to detect the nucleic acid sequence of the invention (especially if the primer is labeled). The invention further encompasses nucleic acid molecules that differ from any of the nucleic acid molecules of the invention in codon sequences due to the degeneracy of the genetic code.

The invention also encompasses nucleic acid sequences or molecules that are analogs of the nucleic acid sequences and molecules described herein. The term "a nucleic acid sequence which is an analog" means a nucleic acid sequence which has been modified as compared to the sequences described herein, such as sequences of (a), (b), (c), (d), or (e), above wherein the modification does not alter the utility of the sequences described herein. The modified sequence or analog may have improved properties over the sequence shown in (a), (b),(c), (d) or (e). One example of a modification to prepare an analog is to replace one of the naturally occurring bases (i.e. adenine, guanine, cytosine or thymidine) of the sequence with a modified base such as such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol

adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8 amino guanine, 8-thiol guanine, 8-thiolalkyl guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines, adenines, or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

Another example of a modification is to include modified phosphorous or oxygen heteroatoms in the phosphate backbone, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages in the nucleic acid molecules of the invention. For example, the nucleic acid sequences may contain phosphorothioates, phosphotriesters, methyl phosphonates, and phosphorodithioates.

A further example of an analog of a nucleic acid molecule of the invention is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in the DNA (or RNA), is replaced with a polyamide backbone which is similar to that found in peptides [37]. PNA analogs have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind stronger to a complimentary DNA sequence due to the lack of charge repulsion between the PNA strand and the DNA strand. Other nucleic acid analogs may contain nucleotides containing polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may have morpholino backbone structures (U.S. Pat. No. 5,034,506). The analogs may also contain groups such as reporter groups, a group for improving the pharmacokinetic or pharmacodynamic properties of nucleic acid sequence.

It will be appreciated that the invention includes nucleic acid molecules encoding truncations of proteins of the invention, and analogs and homologs of proteins of the invention and truncations thereof, as described above. The invention further includes biologically active fragments of the nucleic acid molecules of the invention. Such fragments would include, but is not necessarily limited to any nucleic acid molecules which are beneficial in the modulation or simulation of the interferon receptor binding protein of the invention, or in the identification or production of such agents.

Further, it will be appreciated that the invention includes nucleic acid molecules comprising nucleic acid sequences having substantial sequence homology with the nucleic acid sequences as shown in SEQ. ID. NO. 29 or SEQ. ID. NO.32

(Figure 13) and fragments thereof. The term "sequences having substantial sequence homology" means those nucleic acid sequences that have slight or inconsequential sequence variations from these sequences, i.e., the sequences function in substantially the same manner to produce functionally equivalent proteins. The variations may be attributable to local mutations or structural modifications.

Nucleic acid sequences having substantial homology include nucleic acid sequences having at least 85%, preferably 90-95% identity with the nucleic acid sequences of the invention.

An isolated nucleic acid molecule of the invention that is DNA can be formed and isolated by selectively amplifying a nucleic acid encoding an interferon receptor binding domain of the invention of the invention using the polymerase chain reaction (PCR) methods and cDNA or genomic DNA. It is possible to design synthetic oligonucleotide primers from the nucleic acid sequences of the invention for use in PCR. A nucleic acid can be amplified from cDNA using these oligonucleotide primers and standard PCR amplification techniques. The primers can be designed so that suitable restriction sites are incorporated into the amplified product at its end to enable it to be inserted into the suitable site of the scaffold (scFv) peptide. The nucleic acid sequence encoding the scaffold can also be amplified in a manner to ensure that suitable restriction sites are present at the site of insertion of the interferon receptor binding domain(s). Thus the amplified nucleic acid sequence of the interferon receptor binding domain can be restricted using the appropriate enzymes and then spliced into the analogous restricted site of the scaffold.

The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. It will be appreciated that cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by using the guanidinium-thiocyanate extraction procedure of Chirgwin et al., Biochemistry, 18, 5294 5299 (1979) [38]. cDNA is then synthesized from the mRNA using reverse transcriptase (for example, Moloney MLV reverse transcriptase available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase available from Seikagaku America, Inc., St. Petersburg, FL).

An isolated nucleic acid molecule of the invention that is RNA can be isolated by cloning a cDNA encoding a novel protein of the invention into an appropriate

vector which allows for transcription of the cDNA to produce an RNA molecule which encodes a protein of the invention. For example, a cDNA can be cloned downstream of a bacteriophage promoter, (e.g., a T7 promoter) in a vector, cDNA can be transcribed *in vitro* with T7 polymerase, and the resultant RNA can be isolated by standard techniques.

A nucleic acid molecule of the invention may also be chemically synthesized using standard techniques. Various methods of chemically synthesizing polydeoxynucleotides are known, including solid-phase synthesis which, like peptide synthesis, has been fully automated in commercially available DNA synthesizers (See e.g., Itakura et al. U.S. Patent No. 4,598,049; Caruthers et al. U.S. Patent No. 4,458,066; and Itakura U.S. Patent Nos. 4,401,796 and 4,373,071).

Determination and confirmation of whether a particular nucleic acid molecule encodes a novel protein of the invention may be accomplished by expressing the cDNA in an appropriate host cell by standard techniques, and testing the activity of the protein using the methods as described herein. A cDNA having the activity of a novel protein of the invention so isolated can be sequenced by standard techniques, such as dideoxynucleotide chain termination or Maxam-Gilbert chemical sequencing or by automated DNA sequencing, to determine the nucleic acid sequence and the predicted amino acid sequence of the encoded protein.

The sequence of a nucleic acid molecule of the invention may be inverted relative to its normal presentation for transcription to produce an antisense nucleic acid molecule. The term "antisense" nucleic acid molecule is a nucleotide sequence that is complementary to its target. Preferably, an antisense sequence is constructed by inverting a region preceding or targeting the initiation codon or an unconserved region. In another embodiment the antisense sequence targets all or part of the mRNA or cDNA of the nucleic acid sequences of the invention. In particular, the nucleic acid sequences contained in the nucleic acid molecules of the invention or a fragment thereof, preferably a nucleic acid sequence shown in SEQ. ID. NO. 29 or 32 (Figure 13) may be inverted relative to its normal presentation for transcription to produce antisense nucleic acid molecules.

The antisense nucleic acid molecules of the invention or a fragment thereof, may be chemically synthesized using naturally occurring nucleotides or variously

modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed with mRNA or the native gene e.g. phosphorothioate derivatives and acridine substituted nucleotides. The antisense sequences may be produced biologically using an expression vector introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense sequences are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the vector is introduced.

The invention also provides nucleic acids encoding fusion proteins comprising a novel protein of the invention and a selected protein, or a selectable marker protein

The length and bases of primers for use in a PCR are selected so that they will hybridize to different strands of the desired sequence and at relative positions along the sequence such that an extension product synthesized from one primer when it is separated from its template can serve as a template for extension of the other primer into a nucleic acid of defined length. Primers which may be used in the invention are oligonucleotides, i.e., molecules containing two or more deoxyribonucleotides of the nucleic acid molecule of the invention which occur naturally as in a purified restriction endonuclease digest or are produced synthetically using techniques known in the art such as for example phosphotriester and phosphodiester methods [See 39] or automated techniques [See for example 40]. The primers are capable of acting as a point of initiation of synthesis when placed under conditions which permit the synthesis of a primer extension product which is complementary to a DNA sequence of the invention, i.e., in the presence of nucleotide substrates, an agent for polymerization such as DNA polymerase and at suitable temperature and pH. Preferably, the primers are sequences that do not form secondary structures by base pairing with other copies of the primer or sequences that form a hair pin configuration. The primer preferably contains between about 7 and 25 nucleotides.

The primers may be labelled with detectable markers which allow for detection of the amplified products. Suitable detectable markers are radioactive markers such as P-32, S-35, I-125, and H-3, luminescent markers such as chemiluminescent markers, preferably luminol, and fluorescent markers, preferably dansyl chloride, fluorcein-5-isothiocyanate, and 4-fluor-7-nitrobenz-2-axa-1,3

diazole, enzyme markers such as horseradish peroxidase, alkaline phosphatase, ß-galactosidase, acetylcholinesterase, or biotin.

It will be appreciated that the primers may contain non-complementary sequences provided that a sufficient amount of the primer contains a sequence which is complementary to a nucleic acid molecule of the invention or oligonucleotide fragment thereof, which is to be amplified. Restriction site linkers may also be incorporated into the primers allowing for digestion of the amplified products with the appropriate restriction enzymes facilitating cloning and sequencing of the amplified product.

In an embodiment of the invention a method of determining the presence of a nucleic acid molecule having a sequence encoding a protein of the invention is provided comprising treating the sample with primers which are capable of amplifying the nucleic acid molecule or a predetermined oligonucleotide fragment thereof in a polymerase chain reaction to form amplified sequences, under conditions which permit the formation of amplified sequences and, assaying for amplified sequences.

Polymerase chain reaction as used herein refers to a process for amplifying a target nucleic acid sequence as generally described in Innis et al, Academic Press, 1990 [41] in Mullis el al., U.S. Pat. No. 4,863,195 and Mullis, U.S. Patent No. 4,683,202. Conditions for amplifying a nucleic acid template are described in M.A. Innis and D.H. Gelfand, PCR Protocols, A Guide to Methods and Applications M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White eds, pp3-12, Academic Press 1989 [42].

The amplified products can be isolated and distinguished based on their respective sizes using techniques known in the art. For example, after amplification, a DNA sample can be separated on an agarose gel and visualized, after staining with ethidium bromide, under ultra violet (uv) light. DNA may be amplified to a desired level and a further extension reaction may be performed to incorporate nucleotide derivatives having detectable markers such as radioactive labelled or biotin labelled nucleoside triphosphates. The primers may also be labelled with detectable markers as discussed above. The detectable markers may be analyzed by restriction enzyme digestion and electrophoretic separation or other techniques known in the art.

Conditions which may be employed in the methods of the invention using PCR are those which permit hybridization and amplification reactions to proceed in the presence of DNA in a sample and appropriate complementary hybridization primers. Conditions suitable for a polymerase chain reaction are generally known in the art. For example, see [42]. To amplify DNA template strands, preferably, the PCR utilizes polymerase obtained from the thermophilic bacterium Thermus aquatics (Taq polymerase, GeneAmp Kit, Perkin Elmer Cetus) or other thermostable polymerase.

It will be appreciated that other techniques such as the Ligase Chain Reaction (LCR) and NASBA may be used to amplify a nucleic acid molecule of the invention (Barney in "PCR Methods and Applications", August 1991, Vol.1(1), page 5 [43], and European Published Application No. 0320308, published June 14, 1989, and U.S. Serial NO. 5,130,238 to Malek).

Pharmaceutical Compositions of the Invention and Therapeutic Applications

Interferon, especially human Type I interferon, is known to affect a wide variety of cellular functions, related to cell growth control (such as in cancer and various neoplasias), the regulation of immune responses and more specifically, the induction of antiviral responses. As such the peptides of the present invention can be be used to mimic all or some of the effects of native interferons and thus can be used in the treatment of a number of medical conditions in place of interferon. Preferably, the peptides of the present invention have fewer side effects than those of the native cytokine. Examples of medical conditions in which the peptides of the present invention can be used, include without limtiation to cancers, hematological malignancies, hepatitis B or C infections, multiple sclerosis, different arthritides.

The recombinant nucleic acid sequences of the present invention and constructs, that encode and can express the peptides of the invention under suitable conditions can also be used in the treatment and/or therapy of medical conditions as previously stated.

In one embodiment the invention provides a use of the modulating or simulating agents of the invention (which comprise the peptides, nucleic acid molecules, expression vectors and cells expressing the peptides of the invention) for the treatment of a condition wherein IFN treatment is or maybe indicated. Methods of such treatment are also encompassed within the scope of the invention.

Accordingly, the present invention provides a method of treating or preventing a disease. The invention further comprises uses of the peptides and nucleic acid sequences disclosed herein for the preparation of a medicament for treating a condition wherein IFN treatment is indicated, more preferably human Type I IFN. In another embodiment the invention provides a method for treating such a condition comprising administering an agent that modulates or simulates IFN expression or activity to an animal, preferably a mammal, more preferably a human, in need thereof. In a preferred embodiment, preferably such agents stimulate or simulate IFN activity. Examples of agents that activate or simulate IFN activity would include without limitations, IFN, the gene encoding for IFN with suitable promoters, such promoters preferably being tissue specific promoters and therapeutically effective fragments of the nucleic acid and amino acid sequences of the invention.

Accordingly, the invention provides a method for treating a disease or condition wherein IFN treatment is indicated by administering to a patient in need thereof an agent which simulates IFN activity.

Agents that activate, or stimulate IFN can be formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. As used herein "biologically compatible form suitable for administration *in vivo*" means a form of the substance to be administered in which therapeutic effects outweigh any toxic effects. The substances may be administered to animals in need thereof. Animals, as used herein refers to any animal susceptible to a medical condition that can be modulated or treated with IFN preferably mammals, such as dogs, cats, mice, bovine, horses and humans, preferably humans.

Administration of an “effective amount” of pharmaceutical compositions of the present invention is defined as an amount of the pharmaceutical composition, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a substance may vary according to factors such as disease state, age, sex, and weight of the recipient, and the ability of the substance to elicit a desired response in the recipient. Dosage regima may be adjusted to provide an optimum Therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

An active substance may be administered in a convenient manner such as by injection (subcutaneous, intravenous, topical, intratumoral etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active substance may be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

The compositions described herein can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle or carrier . Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences [44]. On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

Recombinant nucleic acid molecules comprising a sense, an antisense sequence or oligonucleotide fragment thereof, may be directly introduced into cells or tissues *in vivo* using delivery vehicles known in the art such as retroviral vectors, adenoviral vectors and DNA virus vectors. They may also be introduced into cells *in vivo* using physical techniques known in the art such as microinjection and electroporation or chemical methods such as coprecipitation and incorporation of DNA into liposomes. Recombinant molecules may also be delivered in the form of an aerosol or by lavage.

In another embodiment, the peptide, the peptide mimetics of the invention can be used to screen for modulators of the IFN activity. For instance, the mimetic of the invention and its substrate (e.g. Ifn receptor) can be incubated in the presnce of the potential modulator and the effect on mimetic/substrate binding can be detected. There are a number of detection systems known in the art such as, assay detection systems, such as colormetric assays or radiolabelling asays. Agonists, antagonists and other modulators can be screened using this or other methods known in the art.

In another embodiment, the peptide mimetic can be used to diagnose IFN related disorders by obtaining sample (such as blood or tissue sample) from a patient,

exposing it it a peptide mimetic and/or a substrate and comparing effect (e.g. effect on receptor binding) with those of otheres with a known IFN disease state (normal, abnormal or both). Alternatively, the peptide mimetic can be administered to a patient and a patient sample taken afterward for subsequent testing by assay or other means of IFN mimetic/subtrate interaction determination.

The following examples are for illustrative purposes only and in no way are intended to limit the scope of the invention described herein.

EXAMPLES

Example 1 - Interferon Receptor Binding Domains

Fish et al in J. IFN Res. (1989) 9, 97-114 [45] have identified three regions in IFN-alpha that contribute toward the active configuration of the molecule. These three regions include: 10-35, 78-107 and 122-166, and more importantly regions 29-35, 78-95 and 130-140.

The structural homology and symmetry observed among a number of haemopoietic cytokine receptors, and specifically the IFN receptors and tissue factor, the membrane receptor for the coagulation protease factor VII, lends support to the functional receptor binding model that was proposed by Bazan, J. F., Pro. Natl. Acad. Sci. (1990) 87, 6934-6938 [46]. This model invokes the presence of a generic binding through that allows recognition of conserved structural elements among different cytokines. The present inventor's data support such a model, at least for the different IFN-alpha molecular species and IFN-beta, since they have identified two conserved elements in the Type 1 IFNs that effect receptor recognition. A third structural element, that is an exposed recognition epitope, confers specificity of cytokine function, including species specificity.

Experiments were conducted using IFNs shown in Table 1 and Figure 14.

TABLE 1

	1	11	21	31
IFN-αCon₁	CDLPQTHSLG	NRRTLILLAQ	MRRISPFS CL	KDRHD FGFPQ
IFN-α2a	CDLPQTHSLG	SRRTLMLLAQ	MRRISLFS CL	KDRHD FGFPQ
(4-155)IFN-α2a	QTHSLG	SRRTLMLLAQ	MRRISLFS CL	KDRHD FGFPQ
4-155(S98)IFN-α2a	QTHSLG	SRRTLMLLAQ	MRRISLFS CL	KDRHD FGFPQ
4-155(L98)IFN-α2a	QTHSLG	SRRTLMLLAQ	MRRISLFS CL	KDRHD FGFPQ
(ESML)IFN-α2a	CDLPETHSLG	SRRTLMLLAQ	MRRISLSS CL	MDRHD FGFPQ
(A30,32,33)IFN-α2a	CDLPQTHSLG	SRRTLMLLAQ	MRRISLFS CA	KAAHD FGFPQ
IFN-α1Nδ4	ETHSLD	NRRTLMLLAQ	MSRISPSS CL	MDRHD FGFPQ
IFN-β	MSYNLLGFLQRSS	NFQCCKLLWQ	LNGRLEY CL	KDRMN FDIPE
MuIFN-αCon	CDLPQTHNLR	NKRALTLLVQ	MRRLSPLS CL	KDRKD FGFPQ

	41	51	61	71
IFN-αCon₁	EEFDGNQFQK	AQAISYLHEM	IQQTFNLFST	KDSSAAWDES
IFN-α2a	EEF-GNQFQK	AETIPVLHEM	IQQIFNLFST	KDSSAAWDET
(4-155)IFN-α2a	EEF-GNQFQK	AETIPVLHEM	IQQIFNLFST	KDSSAAWDET
4-155(S98)IFN-α2a	EEF-GNQFQK	AETIPVLHEM	IQQIFNLFST	KDSSAAWDET
4-155(L98)IFN-α2a	EEF-GNQFQK	AETIPVLHEM	IQQIFNLFST	KDSSAAWDET
(ESML)IFN-α2a	EFF-GNQFQK	AETIPVLHLM	IQQIFNLFST	KDSSAAWDET
(A30,32,33)IFN-α2a	EEF-GNQFQK	AETIPVLHLM	IQQIFNLFST	KDSSAAWDET
IFN-α1Nδ4	EEFDGNQFQK	APAISVHLEL	IQQIFNLFTT	KDSSAAWDED
IFN-β	EEIKQLQQFQK	EDAALTUYEM	LQNIFAIFRQ	DSSSTGWNET
MuIFN-αCon	EKVDAQQIQK	AQAIPVLSEL	TQQILNIFTS	KDSSAAWNAT

	81	91	101	111
IFN-αCon₁	LLEKFYTELY	QQLND LEACY	IQEVGVEETP	LMNVDSILAV
IFN-α2a	LLDKFYTELY	QQLND LEACY	IQGVGVTETP	LMKEDSILAV
(4-155)IFN-α2a	LLDKFYTELY	QQLND LEACY	IQGVGVTETP	LMKEDSILAV
4-155(S98)IFN-α2a	LLDKFYTELY	QQLND LEACY	IQGVGVTETP	LLKEDSILAV
4-155(L98)IFN-α2a	LLDKFYTELY	QQLND LEACY	IQGVGVTETP	LMKEDSILAV
(ESML)IFN-α2a	LLDKFYTELY	QQLND EEACY	IQGGVVTETP	LMKEDSILAV
(A30,32,33)IFN-α2a	LLDKFYTELY	QQLND LEACY	IQGVGVTETP	LMKEDSILAV
IFN-α1Nδ4	LLDKFCTELY	QQLND LEACY	MQEERVGETP	LMNADSILAV
IFN-β	IVENLLANVY	HQINH LKTVL	EEKLEKEDFT	RGKLMSSLHL
MuIFN-αCon	LLDSFCNDLH	QQLND LQACL	MQEVGVQEPP	LTQEDSLLAV

	121	131	141	151
IFN-αCon₁	RK YFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
IFN-α2a	RK YFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
(4-155)IFN-α2a	RK YFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLS
4-155(S98)IFN-α2a	RK YFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLS
4-155(L98)IFN-α2a	RK YFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLS
(ESML)IFN-α2a	RK YFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
(A30,32,33)IFN-α2a	RK YFQRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
IFN-α1Nδ4	KK YFRRITLY	LTEKKYSPCA	WEVVRAEIMR	SFSLSTNLQE
IFN-β	KR YYGRILHY	LKAKEYSHCA	WTIVAVEILR	NFYLINRLTG
MuIFN-αCon	RK YFHRITVV	LREKKHSPCA	WEVVRAEVWR	ALSSSANLLA

	161
IFN-αCon₁	RLRRKE
IFN-α2a	SLRSKE
(4-155)IFN-α2a	
4-155(S98)IFN-α2a	
4-155(L98)IFN-α2a	
(ESML)IFN-α2a	SLRSKE
(A30,32,33)IFN-α2a	SLRSKE
IFN-α1Nδ4	RLRRKE
IFN-β	YLRN
MuIFN-αCon	RLSEEKE

The foregoing table illustrates the amino acid sequence alignment of the different Type 1 IFNs. The designation of the various IFNs is shown in the left hand column and the sequence of IFN-beta is aligned with the other IFNs, commencing with residue 4, to achieve the greatest homology. The critical domains comprising residues 29-35, 78-95 and 123-140 are boxed. The letter codes for the amino acids are as

follows: A, ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

The preferred binding domain peptide regions of the invention comprise peptides of specific amino acid sequences. These sequences are:

(i) an amino acid sequence of CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1);

(ii) an amino acid sequence of ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2);

(iii) an amino acid sequence of ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-HIS-GLN-ILE-ASN-HIS (SEQ. ID. NO. 3);

(iv) an amino acid sequence of: TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4);

(v) an amino acid sequence of: TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5);

(vi) an amino acid sequence of: TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); and

(vii) an amino acid sequence of: GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7);

(viii) an amino acid sequence of GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP-LEU(SEQ.ID.NO. 20);

(ix) an amino acid sequence of LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA(SEQ. ID. NO. 21).

The novel peptides of the invention have been incorporated into interferons and suitable scaffolds to establish their claimed utility. The following description will be made in conjunction with experiments using interferons and scaffolds having the novel peptides incorporated therein but the invention is not to be restricted to such interferons or scaffolds.

IFN-alpha2a and the various derivatives were provided by I.C.I. Pharmaceuticals Division of the UK; IFN-alphaCon.sub.1 was supplied by Amgen of the USA and IFN-alpha.sub.1 N.delta.4 was supplied by Schering Plough Corp of the USA.

IFN-alpha2a, (4-155)IFN-alpha2a, 4-155(S98)IFN-alpha2a and 4-155(L98)IFN-alpha2a had specific activities of 2.times.10.sup.8 U/mg protein; (A30,32,33)IFN-alpha2a was inactive in antiviral assays and (ESML)IFN-alpha2a had a specific activity of 7.5.times.10.sup.6 U/mg protein; IFN-alpha Con.sub.1 had a specific activity of 3.0.times.10.sup.9 U/mg protein; and IFN-alpha.sub.1 N.delta.4 had a specific activity of 7.1.times.10.sup.6 U/mg protein.

Example 2 - Assays for Antiviral and Growth Inhibitory Activity

The cell culture used in this example comprised T98G cells which were derived from a human glioblastoma multiforma tumor and which express in culture a number of normal and transformed growth characteristics. These cells may be routinely subcultured as monolayers, in modified minimum essential medium (hereinafter referred to as alpha-MEM), and supplemented with 10% (v/v) fetal calf serum (hereinafter referred to as FCS).

An in vitro assay for antiviral activity was conducted. T98G cells were seeded at a density of 1.5.times.10.sup.5 /ml in 200 .mu.l alpha-MEM supplemented with 10% FCS in 96-well Microtest (trade mark) II tissues culture plates and treated with dilutions of the IFN preparations for 24 hours. At the time of virus innoculation, the IFNs were removed and 10.sup.4 PFU EMCV was added to individual wells in 100 .mu.l alpha-MEM, 2% FCS. After 24 hours, the cells were ethanol (95%) fixed and the extent of EMCV infection was determined by spectrophotometric estimation of viral CPE. The fixed cells were crystal violet (0.1% in 2% ethanol) stained and

destained (0.5M NaCl in 50% ethanol), and the inhibition of virus infection was estimated from absorbance measurements at 570 nm using a Microplate (trade mark) Reader MR600 and a calibration of absorbance against cell numbers. IFN titers were determined using a 50% cytopathic end-point and converted to international units using an NIH IFN-alpha standard (Ga 23-901-527).

An in vitro assay for growth inhibitory activity was conducted. T98G cells were seeded in 96-well Microtest II tissue culture plates at a density of 5.times.10.sup.3 /ml and either innoculated with two-fold serial dilutions of different molecular species of IFN-alpha or left untreated. After incubation, at 37.degree. C. for 96 hours, the cells were ethanol fixed (95%), crystal violet (0.1% in 2% ethanol) stained and destained (0.5M NaCl in 50% ethanol), then growth inhibition was estimated from absorbance measurements of destained cells at 570 nm (using a Microplate Reader MR600 and a-calibration of absorbance against cell numbers).

The results of these experiments are shown in FIG. 1. The values represented are the average of triplicate determinations and exhibited a SE of +/-4%. Whereas IFN-alpha2a, (4-155)IFN-alpha2a, 4-155(S98)IFN-alpha2a and 4-155(L98)IFN-alpha2a demonstrate comparable growth inhibitory activities within the error of the assay, (ESML)IFN-alpha2a and (A30,32,33)IFN-alpha2a do not exhibit antiproliferative activity. Similarly, IFN-alpha.sub.1 N.delta.4 has minimal antiviral activity (7.1.times.10.sup.6 U/mg protein) and no demonstrable antiproliferative activity over the dose range examined.

Example 3 - IFN - Receptor Interactions

The next series of experiments examined IFN-receptor interactions. Labelling was carried out using .sup.125 I using a solid phase lactoperoxidase method. A 100 .mu.l reaction mixture containing 10 .mu.l 3% B-D-glucose, 10 .mu.l hydrated Enzymo-beads (trade mark) (available from BioRad in California, USA) 2 .mu.Ci Na.sup.125 I and 20 .mu.g HuIFN-alpha in PBS, pH 7.2, was reacted overnight at +4.degree. C. Free .sup.125 I was separated from IFN-bound .sup.125 I on a 12 ml Sephadex (trade mark) G-75 column, equilibrated in PBS containing 1 mg/ml BSA. Iodination caused no detectable loss of antiviral activity. Fractions containing maximum antiviral activity were pooled and contained 95% TCA (10%) precipitable radioactivity.

Sub-confluent T98G cell monolayers were incubated at +4.degree. C. in alpha-MEM containing 2% FCS and indicated concentrations of .sup.125 I-IFN-alpha. After 2 hours, the binding medium was aspirated and the cultures were washed twice with ice-cold PBS. The cells were solubilized in 0.5M NaOH and radioactivity counted in a Beckman (trade mark) 5500 *-counter. Specificity of binding was determined in parallel binding assays containing a 100-fold excess of unlabeled growth factor. For competitive experiments, specified amounts of unlabeled competitor were included in the reaction mixture together with radiolabelled ligand.

Specific .sup.125 I-IFN-alpha binding data were used to determine receptor numbers and dissociation constants, K.sub.d. With increasing concentrations of .sup.125 I-ligand in the cellular binding reactions, respective specific binding activities corresponding to each .sup.125 I-ligand concentration was calculated.

In FIG. 2, panel A illustrates the results using .sup.125 I-IFN-alphaCon.sub.1 ; panel B illustrates the results using .sup.125 I-4-155(S98)IFN-alpha2a; and panel C illustrates the results using .sup.125 I-IFN-alpha.sub.1 N.delta.4. Inset into panels A, B and C are the corresponding Scatchard plots. The competitive displacement profiles are shown in panels D, E and F using 10 ng/ml of .sup.125 I-IFN-alphaCon.sub.1, 3.7 ng/ml of .sup.125 I-4-155(S98)IFN-alpha2a and 300 ng/ml of .sup.125 I-IFN-alpha.sub.1 N.delta.4 respectively, with no unlabeled competitor (100% bound) or the indicated concentrations of IFNs. The values shown were obtained by subtracting non-specific counts/min bound from total counts/min bound. Non-specific binding was determined in the presence of a 100-fold excess of unlabeled IFN. The points represent the mean of triplicate cultures and exhibited a S.E. of +/-3%.

In FIG. 3, panel A illustrates the results using .sup.125 I-(4-155)IFN-alpha2a and panel B illustrates the results using .sup.125 I-4-155(L98)IFN-alpha2a. Inset into panels A and B are the corresponding Scatchard plots. The competitive displacement profiles are shown in panels C and D using 20 ng/ml of .sup.125 I-(4-155)IFN-alpha2a and 8 ng/ml of .sup.125 I-4-155(L98)IFN-alpha2a, with no unlabeled competitor (100% bound) or the indicated concentrations of IFNs. The values shown were obtained by subtracting non-specific counts/min bound from total counts/min bound. Non-specific binding was determined in the presence of a 100-fold excess of

unlabeled IFN. The points represent the mean of triplicate cultures and exhibited a S.E. of +/-3%.

FIGS. 2 and 3 illustrate the steady state receptor binding characteristics of the different IFN-alpha molecular species on T98G cells at +4.degree. C. Specific binding to sub-confluent T98G monolayers is resolved into a biphasic Scatchard plot. This IFN binding heterogeneity has been shown to result from negatively cooperative site-site interactions between the ligand receptors. Analysis of the IFN-alpha2a binding data reveals both high and low affinity binding components, with K.sub.d s of 2-3.times.10.sup.-11 M and 2-5.times.10.sup.-9 M, respectively. It was found that .sup.125 I(ESML) IFN-alpha2a exhibited no detectable binding activity on proliferating (log phase) T98G cells at +4.degree. C. .sup.125 I-IFN-alphaCon.sub.1 binding to cells was resolved into high affinity K.sub.d 7.7.times.10.sup.-12 M) and low affinity (K.sub.d 1.4.times.10.sup.-9 M) components as shown in FIG. 2A. Similarly, .sup.125 I-4-155(S98)IFN-alpha2a (FIG. 2B), .sup.125 I(4-155)IFN-alpha2a (FIG. 3A) and .sup.125 I-4-155(L98)IFN-alpha2a (FIG. 3B) exhibited binding heterogeneity on T98G cells, with high and low affinity components comparable to IFN-alpha2a. .sup.125 I-IFN-alpha.sub.1 N.delta.4 binding to T98G cells was resolved into a monophasic Scatchard plot, with a single low affinity binding component of K.sub.d 10.sup.-7 M (FIG. 2C). Indeed, competitive binding studies with either .sup.125 I-IFN-alphaCon.sub.1 (FIG. 2D) or .sup.125 I-IFN-alpha.sub.1 N.delta.4 (FIG. 2F), confirmed that IFN-alpha.sub.1 N.delta.4 has a weaker affinity for the IFN-alpha receptor on T98G cells than IFN-alphaCon.sub.1. Substitution of the cysteine residue at position 98 in IFN-alpha2a with a serine, does not affect the polarity or charge distribution of the side chain at this position (CH.sub.2 --SH to CH.sub.2 --OH), yet substitution with a leucine residue does introduce an aliphatic side chain and hence alter the polarity (CH.sub.2 --SH to CH--(CH.sub.3).sub.2). This alteration in side chain polarity at this residue position is not reflected in altered affinity characteristics for the IFN-alpha receptor (FIG. 3B). As would be anticipated, substitution of the cysteine residue at position 98 with serine, did not affect receptor binding characteristics (FIGS. 2B,E). The data from the competitive binding studies, indicate that the IFN-alpha2a variants (ESML)IFN-

alpha2a and (A30,32,33)IFN-alpha2a, are unable to bind to the IFN-alpha receptor (FIGS. 3C,D).

Example 4 - IFN Structure

Since the amino acid sequence dictates the native conformation of a protein, the inventor has ascribed protein structure for the different IFN-alphas and IFN-beta. Receptor recognition epitopes are characteristically hydrophilic and located on the surface of the binding molecule. Generally, sites for molecular recognition in proteins are located in loops or turns, whereas alpha-helices are involved in maintaining the structural integrity of the protein. Close examination of the hydrophilicity and surface probability plots of IFN-alpha2a shows that, in those regions that are critical for the active configuration of IFN-alpha, namely 10-35, 78-107 and 123-166, altering the cysteine at 98 has no effect on these determinants (FIG. 4), and indeed, does not affect biological activity (FIG. 1).

FIG. 4 illustrates predicted secondary structure characteristics of different IFN-alpha species according to amino acid sequence analyses. Hydrophilicity (H) and surface probability (S) profiles are depicted for each of the IFN-alphas and IFN-beta whose designations are on the left hand side of each pair. Amino acid residue position is indicated along the horizontal axes of the graphs. The critical domains comprising residues 29-35, 78-95 and 123-140 are boxed.

In IFN-alpha2a, in the carboxy-terminal domain there are essentially 3 hydrophilic residue clusters that are likely located on the surface of the molecule (FIG. 4). Deletion of the cluster closest to the carboxy-terminus, in (4-155)IFN-alpha2a, has no effect on antiviral specific activity, growth inhibitory activity (FIG. 1), or receptor binding characteristics (FIG. 3), compared with the full length IFN-alpha2a. Thus, for receptor recognition, the region 155-166 does not influence the active configuration of the previously defined strategic domain 123-166. Interestingly, there are two peaks of hydrophilicity in this carboxy-terminal region, that spans residues 123-140, that correspond to a helical bundle and loop structure. In the human, equine, bovine, ovine, rat and murine IFN-alphas, human and murine IFN-beta, cow trophoblast IFN (TP-1) and horse IFN-omega, all designated Type 1 IFNs, these structural motifs are highly conserved (FIG. 4), lending credence to the notion that this carboxy-terminally located domain is critical for receptor recognition for the

Type 1 IFNs. The alpha-helical structure, that constitutes residues 123-129, allows the appropriate presentation of the loop structure around residues 130-140, and this loop structure serves as a recognition epitope for receptor binding. This conclusion is consistent with reports that the region that comprises residues 123-136 influences biological activities on human and murine cells. Further examination of the 10-35 domain, reveals a single region that is likely located on the surface of the molecule and contains hydrophilic residues, namely 29-35. Other reports have implicated the amino-terminal region of IFN-alpha, in particular amino acid residue 33, as critical for biological activity on human and bovine cells. The IFN-alpha2a variants (A30-32,33)IFN-alpha2a and (E5,S27,M31,L59)IFN-alpha2a, that have lost biological activity and receptor binding characteristics, no longer present this cluster of residues near the surface of the molecule, (FIG. 4). This region constitutes a loop structure. In IFN-alpha.sub.1 N.delta.4, the amino acid residues that immediately precede the critical 29-35 cluster are different to those in IFN-alpha2a, and thus affect the presentation of this receptor binding epitope somewhat, according to the different predictive algorithms the inventor has employed. The data in FIG. 4 suggest that the cluster of hydrophilic residues that do constitute this receptor recognition epitope will be located near the surface of the molecule in IFN-alpha.sub.1 N.delta.4. However, substitution of the lysine residue at position 31 by a methionine residue, affects the configuration of this receptor recognition epitope, thereby affecting the biological effectiveness of IFN-alpha.sub.1 N.delta.4. In the human and murine IFNs, the loop structure that includes residues 29-35, is conserved, yet CLKDRHD is presented as CLKDRMN and NLTYRAD, respectively (see FIG. 3). In murine consensus IFN-alpha, MuIFN-alphaCon, this epitope is conserved as CLKDRKD, where H (histidine) to K (lysine) is a conservative change with respect to side chain group and charge. Considerable sequence homology with the human residues 29-35 is also apparent among the murine, equine, ovine, bovine and rat IFN-alphas, as well as for cow TP-1 and horse IFN-omega. The Type 1 IFNs share conserved receptor recognition epitopes in the 29-35 and 123-140 regions. Some variance is seen in the human and murine IFN-beta in the 29-35 region, although the presentation of this epitope as a loop structure is conserved.

The third strategic region with respect to the active configuration of IFN-alpha spans residues 78-107. A hydrophilic cluster of amino acid residues that are likely located on the surface constitute residues 83-95 (FIG. 4). These residues probably present as a contiguous helical bundle and a loop structure. Several amino acid residues around position 78 also appear to be located at the surface as part of the helical bundle. The inventor has shown that substitution of the cysteine at position 98 with either a serine (S) or a leucine (L) does not affect the receptor binding characteristics of IFN-alpha2a, hence the inventor infers that those residues beyond 95, in the previously defined domain 78-107, are likely not critical for receptor recognition in IFN-alpha, since they appear not to be located at the surface of the molecule. The alpha-helical structure allows the appropriate presentation of the recognition epitope that comprises residues 88-95. Of note is the variance in this region between the human IFN-alphas and the murine IFN-alphas, and the human IFN-alphas had human IFN-beta. Of the three previously defined critical active domains in the Type I IFNs, it is this domain that exhibits the most divergence with respect to species, and alpha-versus beta-IFNs (Table 1). It is noteworthy that the hybrid IFN, IFN-alphaAD(BgI II), exhibits a hydrophilicity plot somewhat different from the human IFN-alphas in this region, yet similar to that seen for the murine IFNs, specifically MuIFN-alphaCon (FIG. 4). Both MuIFN-alphaCon and IFN-alphaAD(BgI II) have a cysteine residue at position 86, in contrast with the majority of human IFN-alphas, for which there is a tyrosine residue in this position. These data are consistent with IFN-alphaAD(BgI II) showing demonstrable biological activity on murine cells and support the hypothesis that this region in the Type I IFNs determines species specificity. Indeed, the hybrid IFN-alphaAD(PvuII) resembles the human IFN-alphas in this region (FIG. 4) and differs from IFN-alphaAD(BgI II) at just three residue positions, two of which reside in this critical domain: 69 (S/T), 80(T/D) and 86(Y/C). IFN-alphaAD(Pvu II) demonstrates considerably reduced antiviral activity on murine cells compared with IFN-alphaAD(BgI II) yet comparable activity to IFN-alpha 2a, on human cells.

Sequence homology among the different Type 1 IFNs in conserved regions would suggest evolutionary significance. It is noteworthy that the amino-and carboxy-terminal domains that have been identified as critical, are highly conserved among the

different molecular subtypes of Type 1 IFNs. Within the 29-35 and 123-140 regions are structural motifs that are consistent with receptor binding domains: loop structures that are predominantly hydrophilic and located at the surface of the molecule. Some variation in sequence homology is apparent in the 78-95 region. The critical epitopes for Type I IFN receptor recognition are associated with the residue clusters 29-35 and 130-140, for all species of Type I IFNs. These epitopes constitute the receptor binding domains and are likely located in close spacial proximity to one another in the folded IFN. The specificity of action of a particular Type I IFN is conferred by the recognition epitope 78-95.

The basis for the specificity of interaction of the 78-95 domain and its putative cognate binding molecule is unknown. Studies with human growth hormone have shown that receptor binding involves both receptor recognition, by an epitope on the growth hormone, and dimerization of receptors, facilitated through the interaction of a separate epitope on the growth hormone. By analogy, once an IFN-alpha molecule is bound to its receptor, mediated by the recognition epitopes 29-35 and 130-140, the 78-95 epitope in HuIFN-alpha may interact with another Type 1 receptor, effecting dimerization. Using the cross-linking agent disuccinimidyl suberate for analysis of affinity- labeled cellular IFN binding components, the inventor and a number of other groups have shown that IFN-receptor complexes of 80 kDa and 140-160 kDa can be separated by SDS-PAGE. The molecular weight of the predicted IFN-alpha receptor protein is 63 kDa and that of the majority of IFN-alphas is 20 kDa, thus, monomer (receptor-IFN) and dimerized-(receptor-IFN-receptor) complexes, may represent the 80 kDa and 40-160 kDa moieties that have been detected.

FIG. 5 illustrates a model for the tertiary structure of Type 1 IFNs. This model incorporates a helical bundle core, composed of the five helices A to E. The loop structures that constitute the proposed receptor recognition epitopes, residues 29-35 and 130-140, are shown as heavily shaded, broad lines and are aligned such that they dock in the receptor groove as shown. The third region implicated in the active conformation of the Type 1 IFNs, 78-95, is not buried in the receptor groove and is configured to allow binding to its cognate epitope on another Type 1 IFN receptor. The shaded areas in helices C and D represent residues that are critical for maintaining the correct structural presentation of the corresponding contiguous

recognition epitopes. In agreement with a number of different models that have been proposed, the Type I IFNs are comprised predominantly of alpha-helical bundles that are packed together. The receptor recognition site is comprised of the AB loop, 29-35 and the D helix and DE loop, 123-140. These are aligned in such a way as to permit the IFN to bind to its receptor, in the receptor groove, such that the third epitope, 78-95, is exposed and not buried in the receptor groove. The initial interaction of the IFN molecule with the Type I IFN receptor would account for the abundant, low affinity receptor binding component, extrapolated from the Scatchard analyses of the different binding isotherms. The higher affinity component could be invoked once the IFN molecule is bound to its receptor. The heterogeneity of binding observed for IFN-alpha2a is absent in IFN-alpha.sub.1 N.delta.4, and is explained by the alteration of the 29-35 and 78-95 epitopes in IFN-alpha.sub.1 N.delta.4, as compared with IFN-alpha2a. This may lead to a reduction in signaling potential of the receptor-bound IFN and hence a reduction in biological potency.

There is some evidence to suggest that the proliferative state of a cell will determine whether the high affinity binding component is invoked on IFN-alpha2a binding to its receptor. Non-proliferating cells express fewer Type I IFN receptors and will not exhibit the characteristic heterogeneity of binding seen with proliferating cells. Interestingly, non-proliferating cells do possess both the 80 kDa and 140-160 kDa IFN-binding complexes. The data indicate that non-proliferating cells lack the high affinity component of IFN-alpha binding, that is not associated with IFN-receptor dimerization, yet may represent a secondary binding molecule. A comprehensive binding model, therefore, that would account for heterogeneity of binding distinct from receptor dimerization, would invoke the interaction of the IFN-bound receptor complex with a putative secondary binding molecule. The possibility that other accessory molecules are required for the full complement of IFN-receptor interactions, is supported by observations of high molecular weight complexes containing the IFN-alpha-receptor complex. Furthermore, the genetic transfer of the human IFN-alpha receptor into mouse cells, led to transfectants that exhibited a poor sensitivity to selected Type 1 human IFNs. These results infer that the transfected protein may not be sufficient for the complete binding activities of the IFNs. Indeed, in the receptor systems described for interleukin-6 and nerve growth factor, accessory

proteins are required for the high affinity binding component of the receptor-ligand interaction. In the absence of experimental data, it cannot be discounted that the 78-95 epitope in Type 1 IFNs may interact with a species-specific secondary binding molecule. It is intriguing to suggest that the differential specificity of action that resides in IFN-alpha and IFN-beta, results from the specific interaction of the 78-95 region in the two IFNs with a complementary cognate accessory binding molecule. Moreover, the species specificity observed for the Type 1 IFNs may reside in the recognition of this species-specific cognate binding molecule, by the specific and variable 78-95 epitopes amongst the different Type 1 IFN species. The precedent for major determinants of specificity of interaction has been made with small nuclear ribonucleoproteins and specific RNAs: RNA binding specificity is conferred by short stretches of variant amino acid residues in two ribonucleoproteins that otherwise share extensive sequence homology. Certainly, among DNA binding proteins, exchange of amino acid residues between members of the helix-turn-helix and zinc finger protein families can result in the exchange of DNA binding specificity. The nature of the accessory binding molecule that may be associated with the Type 1 IFN receptor complex remains to be clarified.

Example 5 - Preparation of Biological Scaffold, Modeling of C219scfv

The structural coordinates of C219scFv and the interferons , such as IFN-alpha2b can be found at URL http://www.rscb.org/pdb (the Protein Data Bank) as follows:

C219scFv accession number: 1AP2

IFN-a2b accession number: 1RH2 (alpha carbon co-ordinates are available)

The modeling of the biological scaffold was prepared using Insight II molecular modeling software on a Silicon Graphics Unix platform.

Figure 8A illustrates a consensus interferon alpha carbon trace showing the tertiary structure of interferon alpha and three IFN binding domains and Figure 8B is a consensus C219scFv carbon trace, wherein the CDRs in the variable light chain region are highlighted. Protein structures of Consensus interferon and C219scfv were superimposed and rotated in three dimensions to determine how the IFN sequences would be placed in the scFv sequence. C219 was developed as a murine monoclonal antibody specific for Chinese hamster P-glycoprotein [47]. A single chain variant was

later developed [48], which variant was engineered by the inventor to result in the scaffold of the present invention. The goal was to reproduce as closely as possible the three-dimensional arrangement seen in the interferon structure. The computational approach allows quick, inexpensive experimentation with different arrangements of loops. The software suite InsightII has the capability for testing various insertion sites on the framework both visually and with energy minimization protocols. The software was run on the OCI Research Unix Network, consisting of mainly Silicon Graphics and Sun workstations. The interferon structure was available to the inventor and used as a guide. Various likely locations for the insertion were sampled in comparison with the relative disposition of the epitope sequences on the native interferon molecule. The advantage of using the scFv scaffold, as opposed to a single-domain framework, for example, is that a number of loop regions were available as potential insertion sites. These included both the remaining complementarity-determining regions and parts of the constant framework region that might be favourably disposed to receive the helical sequence. Preliminary modelling, suggested that the 29-35 sequence could be inserted into the CDR3 loop, the 123-140 sequence replace residues in the CDR1 loop and residues 78-95 could be positioned in the CDR2 region.

Example 6 - Preparation of Scaffold/IFN Binding Domain Constructs and pIFNscFV

Once the modeling of the scFv was accomplished, the constructs of the invention were prepared. Restriction sites were engineered into the CDR loops of variable light chain of scFv using a Stratagene mutagenesis kit. An AccI site was introduced into CDR1 of scFv using an AccI sense primer of SEQ. ID NO. 23 and AccI antisense primer of SEQ. ID NO. 24. These primers were designed with a mutation at nucleotides 20 and 21 in the sense primer, and at nucleotides 21 and 22 in the antisense primer, as shown. The mutation serves to introduce the AccI restriction site, which is engineered into the sequence in order to insert the nucleic acid sequence of SEQ. ID NO. 28, which translates to the interferon-receptor binding peptide of amino acid sequence SEQ. ID NO. 21.

An HpaI site was introduced into the CDR3 of scFv using an HpaI sense primer of SEQ. ID NO. 25, and HpaI antisense primer of SEQ. ID NO. 26. These

primers were designed with a mutation at nucleotides 20 and 22 of the sense primer, and nucleotides 19 and 21 of the antisense primer, as shown. The mutation serves to introduce the HpaI restriction site, which is engineered into the sequence in order to insert the nucleic acid sequence of SEQ. ID NO. 27, which translates to the interferon-receptor binding peptide of amino acid sequence SEQ. ID NO. 1.

cDNAs corresponding to conserved interferon-receptor binding peptides (SEQ. ID NO. 1, SEQ. ID NO. 20, SEQ. ID NO. 21) were synthesized by known methods. DNA was purified by polyacrylamide gel electrophoresis (PAGE) using known methods. Strands of DNA were annealed to produce double stranded coding sequence.

DNA sequences corresponding to interferon-receptor binding peptides (SEQ. ID NO. 1, SEQ. ID NO. 20, SEQ. ID NO. 21) were ligated into the engineered scFv sequence using the appropriate restriction sites. Flanking sequences were also added to increase the stability of the protein secondary structure. The CDR1 insert (SEQ. ID NO. 21) was screened by redigestion with AccI and KpnI. Presence of the CDR3 insert (SEQ. ID NO. 1) was determined by length of the construct using PCR technology and primers as provided in SEQ. ID NO. 30 and SEQ. ID NO. 31. The scFv containing the insert of SEQ. ID NO. 1 should be 93 base pairs in length. Without the insert, the PCR product will be 63 base pairs. To verify that the appropriate inserts were accomplished, sequencing was performed to detect any mutations.

First a partial IFN-scFV (pIFNscFv) was made comprising inserts at CDR1 (With SEQ. ID. NO. 21) and CDR3 (with SEQ. ID. NO. 1)

TG1 E. coli cells were transformed with pSJF2 plasmid containing pIFNscFv (scaffold with inserted interferon-receptor binding sequences) (complete sequence of plasmid containing pIFNscFv is provided in SEQ. ID NO. 22 (for amino acid sequence) and SEQ ID NO. 29 (for nucleic acid sequence) and plated on LB-amp plates. Resulting colonies were picked and grown in 25mL LB containing ampicillin. The periplasmic cell fraction was isolated and screened for the presence of pIFNscFv using an anti-c-Myc antibody and Western blot technology. One positive clone was grown up in large cultures and periplasmic fraction isolated. This fraction was

concentrated using an Amicon system under nitrogen gas with a 10kDa cutoff membrane.

The concentrated periplasmic fraction was dialysed against 10mM Tris pH 8.0, 300mM NaCl and loaded onto a Ni-agarose column. Protein was eluted using 100mM imidazole in dialysis buffer. Fractions were analyzed on Western blot using anti-c-Myc antibody for presence of pIFNscFv. The results are shown in Figure 6B.

Fractions containing scFv from Ni column purification were pooled and dialysed against 50mM Tris pH8.0 (Figures 6A and 6B). Protein was further purified on a Q-column (Figure 7).

Example 7 – Bioactivity of pIFN-scFv

To test the bioactivity of pIFN-scFv, an antiproliferative assay was conducted. Two fold serial dilutions of pIFNscFv in growth meida were added to individual wells of a 96-well tissue culture plate (Sarstedt). IFN-Con was added to each well being treated at indicated amounts (See Figures 9A and 9B). 3 $x10^3$ Daudi Cells were introduced to each well. The cultures were then incubated at $37^{O}C$ at 5% CO_2 humidification for 96 hours. At end of incubation period, an MTT assay (Boehringer Mannheim) was performed in accordance with manufacturer instructions for all culture conditions. Relative to control of untreated cells, the percentage of growth inhibition owas calculated. The results are shown in Figures 9A and 9B. The data demonstrate that addition of C219scFv alone to Daudi cells does not affect their growth. However, addition of the pIFNscFv appears to inhibit the growth-regulatory effects of endogenously produced IFN in Daudi cells (Fig. 9A). Figure 9B demonstrates that at high doses of IFN, that exhibit maximal growth inhibitory activity, competition with the partial mimetic, pIFNscFv, results in soma ablation of the IFN-induced growth inhibitory effects at the highest doses of competitor mimetic.

Example 8 – Preparation of Complete IFNscFV mimetic (cIFNscFV)

The construct the complete mimetic, that contains IFN IRRP sequences from all three binding domains inserted to the corresponding three CDR regions of scFV, the IRRP sequences of IFN-α2 were highlighted and the molecule was manually docked on the C219scFv structure to determine the optimum conformational fit for IRRP placement within the CDRs. In the cIFNscFv, the scaffold needs to accommodate three IRRPs as opposed to two as in the pIFNscFv construct. As a

result of the modeling and structure analysis, the construct encoded by the nucleotide sequence of Figure 13 (SEQ. ID. NO. 32) encoding the amino acid sequence also shown in Figure 13 (SEQ. ID. NO. 33) was constructed. It should be noted that the amino acid 362 (Ser 362) in Figure 13 is not present in theTG1 E.Coli expressed cIFNscFv constructed in this example. However, it is present in the original c219scFv protein. In the c219scFv protein databank file (See URL above), the co-ordinates for Ser362 Gly363 Ser364 are not reported in the B chain. However, in the D chain, these coordinates are reported. The B chain was used in the present structural modeling studies and so. The Ser362 was not include in the present cIFNscFv cDNA sequnce but the Gly363 and Ser362 were included since they comprise a unique BanHI cloning site. . In another embodiment, amino acid 362 can made with or be reinserted into the protein.

First the scFv scaffold was prepared by engineering restrictions sites by known methods in the art, into the CDR loops of variable light chain of scFv using a Stratagene mutagenesis kit. Figure 11 is a plasmid map showing the restirictions introduced into the construct of the scFv scaffold. Figure 11 is a plasmid map of C219 scFv-IFN (cIFNscFv) in pSJF2 vector. The construct was directionally cloned from BsiWI to BanHI. The numbers beside the restriction sites refer to nucleotide numbers within the construct. Figure 15 includes the nucleic acid (SEQ. ID. NO. 18) and amino acid (SEQ. ID. NO. 19) sequences of C219scFv. C219scFv begins at amino acid position 26 of the Figure. The sequence 5' to the scFv protein codes for the OmpA sequence. Amino Acid 278-288 is the c-Myc tag, the His tag follows.

The scFV coding region was then changed as indicated in Figure 10. The nucleotide sequence (SEQ. ID. NO. 42) encoding for the CDR3 loop of the VH domain (SEQ. ID. NO. 45) was replaced with a nucleotide sequence (SEQ. ID. NO. 36) comprising encoding sequence for IFN residues 122-157 (SEQ. ID. NO. 39) that includes IRRP3 sequence. A polyglycine (G_4) tract was added to the C-terminal of the 122-157 IFN sequence to allow a flexible link between the IFN and antibody sequences. The nucleotide sequence (SEQ. ID. NO. 41) near the CDR1 coding region of the VH domain was replaced with a nucleotide sequence (SEQ. ID. NO. 35) for IFN residues 29-37 (SEQ. ID. 38), that includes the IRRP1 sequence 29-35. To properly position the IRRP1 sequence in relation to IRRP3, residues 27-32 were

removed from the C219 sequence, a poly-glycine (G_3) linker was added to the C-terminal of IRRP1 and a 15 Å $(GGGGS)_3$ linker was added to the N-terminal of IRRP1. The nucleotide sequence (SEQ. ID. NO. 40) encoding for the CDR2 loop of the VL domain (SEQ. ID. NO. 43) was replaced with a nucleotide sequence (SEQ. ID. NO. 34) encoding an amino acid sequence for IFN residues 55-97 (SEQ. ID. NO. 37), including the IRRP1 sequence 78-95 Two polyglycine (G_4) linkers were added to join the IRRP2 sequence to the C219scFv sequence. Co-ordinates were assigned to the poly-glycine tracts using the HOMOLOGY module of InsightII, energy minimization was carried out using the DISCOVER module of INSIGHT II and molecular dynamics were also performed on the protein structure. The cDNA was ordered from the Midland Texas Reagent Company in Midland Texas.

The cIFNscFv vector construct (pSJF2-cIFNscFv), was espressed in TG1 cells with and without IPTG as indicated in Figure 12. An anti-c-Myc Western immunoblot of whole cell lysates was used to screen for the presence of the c-Myc tagged cIFNscFv mimetic. An IPTG band, which corresponds to the expected molecular weight of the cIFNscFv mimetic is present in lane 4. The band indicates that the product is about 37kDa. Two bands can actually be seen on the immunoblot, the higher band corresponds tounprocessed protein, i.e., signal sequence is still present.

<u>Summary of cIFNscFv construction</u>

By definition

VL nucleotide sequences and amino acid sequences of:

<u>CDR1</u>

AAG TCC AGT CAG AGT CTG TTA AAA CGT GGA AAT CAA AAG AAC TAC TTG ACC (SEQ. ID. NO. 46)

Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu Thr (SEQ. ID. NO. 47)

<u>CDR2</u>

TGG GCA TCC ACT AGG GAA TCT (SEQ. ID. NO. 48)

Trp Ala Ser Thr Arg Glu Ser (SEQ. ID. NO. 43)

<u>CDR3</u>

TGT CAG ATG TAT AGT TAT CCG (SEQ. ID. NO. 49)

Cys Gln Met Tyr Ser Tyr Pro (SEQ. ID. NO. 50)

VH nucleotide sequences and amino acid sequences of:

<u>CDR1</u>

GGC TTT AAC ATT AAA GAC GAC TTT TAG CAC (SEQ. ID. NO. 51)
Gly Phe Asn Ile Lys Asp Asp Phe Met His (SEQ. ID. NO. 44)

CDR2
AGG ATT GAT CCT GCG AAT GAT AAT ACT AAA TAT GCC CCG AAG TTC CAG GAC (SEQ. ID. NO. 52)
Arg Ile Asp Pro Ala Asn Asp Asn Thr Lys Tyr Ala Pro Lys Phe Gln Asp (SEQ. ID. NO. 53)

CDR3
AGA GAG GTT TAT AGT TAC TAT AGT CCC CTC GAT GTC (SEQ. ID. NO. 54)
Arg Glu Val Tyr Ser Tyr Tyr Ser Pro Leu Asp Val (SEQ. ID. NO. 45)

To generate the cIFNscFv sequence:

CDR2 sequence and some flanking sequences in VL were removed from C219scFv
CTG TTG ATT TAC TGG GCA TCC ACT AGG GAA TCT GGG GTC CCT GAT (SEQ. ID. NO. 40)
Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp (SEQ. ID. NO. 55)

and the following IFN-Con IRRP2 sequence as some flanking sequences were added (non IFN sequences are bolded)
GGA GGC GGC GGG TCT GTG CTT CAC GAA ATG ATT CAG CAG ACC TTT AAT TTA
Gly Gly Gly Gly Ser Val Leu His Glu Met Ile Gln Gln Thr Phe Asn Leu
TTT TCG ACC AAG GAT TCG AGC GCT GCG TGG GAT GAG AGC CTG CTG GAG AAA
Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Ser Leu Leu Glu Lys
TTT TAC ACG GAA CTC TAT CAG CAG CTC AAT GAT CTA GAG **GGT GGA GGT GGC (SEQ. ID. NO. 34)**
Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu **Gly Gly Gly Gly (SEQ. ID. NO. 56)**

Part of CDR1 sequence in VH was removed from C219scFv
TTT AAC ATT AAA GAC GAC (SEQ. ID. NO. 41)
Phe Asn Ile Lys Asp Asp (SEQ. ID. NO. 57)

and the following IFN-Con IRRP1 sequence and some flanking sequences were added (non IFN sequences are bolded)

GGC GGC GGG AGC GGC GGC GGC GGC TCG GGC GGT GGA GGT TCT TGT CTG AAA

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Cys Leu Lys

GAT CGC CAT GAC TTC **GGT GGC GGC (SEQ. ID. NO. 35)**

Asp Arg His Asp Phe **Gly Gly Gly (SEQ. ID. NO. 58)**

Part of CDR3 sequence in VH was removed from C219scFv

GTT TAT AGT TAC TAT AGT CCC CTC GAT (SEQ. ID. NO. 42)

Val Tyr Ser Tyr Tyr Ser Pro Leu Asp (SEQ. ID. NO. 59)

and the following IFN-Con IRRP3 sequence and some flanking sequences were added (non IFN sequences are bolded)

GGC TAT TTC GAC CGT ATT ACC CTG TAC TTA ACC GAG AAA AAA TAC TCC CCA

Gly Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro

TGC GCG TGG GAA GTA GTG CGG GCA GAA ATC ATG CGT TCG TTC AGT TTG TCT

Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser

ACA AAC CTG GGC GGA GGT GGA (SEQ. ID. NO. 36)

Thr Asn Leu Gly Gly Gly Gly (SEQ. ID. NO. 60)

Having illustrated and described the principles of the invention in a preferred embodiment, it should be appreciated to those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. For instance, although the invention has been described in relation to human Type I interferons, human Type I interferon receptor binding domains and peptides and a particular scFv scaffold, a similar methodology and techniques would be equally applicable to other cytokines, cytokine receptors. We claim all modifications coming within the scope of the following claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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FIGURE LEGENDS

FIG. 1

Growth inhibitory activities of variant IFN-.αs in T98G cells.

Cells were incubated with the different IFN-α species, at the indicated doses, at 37°C for 96 hr, then growth inhibition was estimated by spectrophotometric determination, as described.

Values represent the average of triplicate determinations and exhibited a SE of ± 4%. □ IFN-α2a; ■ (4-155)IFN-α2a; △ 4-155(S98)IFN-α2a; ▲ 4-155(L98)IFN-α2a; ◊(ESML)IFN-α2a; ♦ (A30,32,33)IFNα2a.

FIG. 2

Receptor binding characteristics of variant IFN-.αs on T98G cells.

Binding isotherms. 3.5×10^5 T98G cells were incubated for 2 hr at +4°C with the indicated concentrations of ^{125}I-IFN-.αCon$_1$, (A), ^{125}I-4-155(S98)IFN-$_{\_2a}$, (B), or ^{125}I-IFN-α1Nδ4 (C). Inset into A, B and C are the corresponding Scatchard plots.

Competitive displacement profiles. 3.5×10^5 T98G cells were incubated at +4°C for 2 hr with 10 ng/ml ^{125}I-IFN-αCon$_1$, (D), 3.7 ng/ml ^{125}I-4-155(S98)IFN-α2a, (E), or 300 ng/ml ^{125}I-IFN-α1Nδ4, (F), containing no unlabeled competitor (100% bound) or the indicated concentrations of IFNs.

For D and F: ■ IFN-αCon$_1$; □ ^{125}I-IFN-α1Nδ4.

For E: : ■ IFN-α2a; □ 4-155(S98)IFN-α2a; △ 4-155(L98)IFN--α2a.

The values shown were obtained by subtracting non-specific counts/min bound from total counts/min bound. Non-specific binding was determined in the presence of a 100-fold excess of unlabeled IFN. The points represent the mean of triplicate cultures and exhibited a S.E. of ±3%.

FIG. 3

Receptor binding characteristics of variant IFN-.alpha.s on T98G cells.

Binding isotherms 3.5×10^5 T98G cells were incubated for 2 hr at +4°C with the indicated concentrations of ^{125}I-(4-155)IFN-α2a, (A), and ^{125}I-4-155(L98)IFN-α2a, (B). Inset into A and B are the corresponding Scatchard plots.

Competitive displacement profiles

$3.5x10^5$ T98G cells were incubated at +4°C for 2 hr with 20 ng/ml ^{125}I-(4-155)IFN-α2a, (C), or 8 ng/ml ^{125}I-4-155(L98)IFN--α2a, (D), containing no unlabeled competitor (100% bound) or the indicated concentrations of IFNs.

■ IFN--α2a; □ (4-155)IFN--α2a; △ 4-155(L98)IFN-α2a; ▲ (ESML)IFN-α2a; ◊ (A30,32,33) IFN-α2a.

The values shown were obtained by subtracting non-specific counts/min bound from total counts/min bound. Non-specific binding was determined in the presence of a 100-fold excess of unlabeled IFN. The points represent the mean of triplicate cultures and exhibited a S.E. of ±3%.

FIG. 4

Predicted secondary structure characteristics of different IFN-.α species according to amino acid sequence analyses.

Hydrophilicity, H, and surface probability, S, profiles are depicted for each of the IFN-αs and IFN-β, whose designations are on the left hand side of each pair. Amino acid residue position is indicated along the horizontal axes of the graphs. The critical domains, comprising residues 29-35, 78-95 and 123-140, are boxed.

FIG. 5

Model for the tertiary structure of Type I IFNs. This model incorporates a helical bundle core, composed of the 5 helices A-E. The loop structures that constitute the proposed receptor recognition epitopes, residues 29-35 and 130-140, shown here as heavily shaded, broad lines, are aligned such that they dock in the receptor groove as shown. The third region implicated in the active conformation of the Type I IFNs, 78-95, is not buried in the receptor groove and is configured to allow binding to its cognate epitope on another Type 1 IFN receptor. The shaded areas in helices C and D represent residues that are critical for maintaining the correct structural presentation of the corresponding contiguous recognition epitopes (see text).

FIG. 6

Coomassie stain (A) and corresponding Western blot (B) of periplasmic extract purified on a Ni-agarose column. Purified C219scFv was used as a positive control. The Load flo-thru lane indicates protein that is not binding to the column. 6mM imidazole washes elute protein species that have bound non-specifically to the column. The anti-c-Myc Western blot shows the expected IFNscFv band at approximately 40kDa. Fractions collected during elution with buffer containing 100mM Imidazole are numbered 1 through 10. 16μL from 2mL fractions were resolved in 12% SDS-polyacrylamide gels.

FIG. 7

Silver stain of protein fractions (numbered 10-19) eluted from Q-column purification. A NaCl gradient (0M to 1M) was used to elute the proteins. The bands corresponding to the IFNscFv protein, at a molecular weight of approximately 40kDa, in fractions 10 to 14, are boxed. 16mL from 2mL fractions were resolved in a 12% SDS-polyacrylamide gel.

FIG. 8

FIG. 8A is a consensus interferon alpha carbon trace showing the tertiary structure of interferon alpha and three IFN binding domains. FIG. 8B is a consensus C219scFv carbon trace, wherein the CDRs in the variable light chain region are highlighted.

What is claimed is:

1. A cytokine receptor binding peptide construct comprising at least one cytokine receptor binding domain incorporated in a suitable molecular scaffold such that the scaffold maintains the binding domain in a configuration suitable for binding to the cytokine receptor.

2. The cytokine receptor binding peptide construct of claim 1 wherein the scaffold is an immunoglobulin fragment comprising the variable region.

3. The cytokine receptor binding peptide construct of claim 2 wherein the immunoglobulin fragment is a scFv molecule.

4. The cytokine receptor binding peptide construct of claim 3 wherein the scFv molecule is a C219 scFv molecule

5. The cytokine receptor binding peptide construct of claim 4, wherein the scFv molecule has three complimentarity determining regions: CDR1, CDR2 and CDR3.

6. The cytokine receptor binding peptide construct of claim 5 wherein at least one cytokine receptor binding domaine is incorporated into the scFv molecule in at least one of the complimentarity determining regions, altering the respective complimentarity determining region.

7. An interferon receptor binding peptide construct comprising at least one interferon receptor binding domain incorporated in a suitable molecular scaffold such that the scaffold maintains the binding domain in a configuration suitable for binding to the interferon receptor.

8. The interferon receptor binding peptide construct of claim 7 wherein the interferon is Type I human interferon.

9. The interferon receptor binding peptide construct of claim 8 wherein the scaffold is a scFv molecule.

10. The interferon receptor binding peptide construct of claim 9 wherein the scFv molecule has three complimentarity determining regions: CDR1, CDR2 and CDR3.

11. The inteferon receptor binding peptide construct of claim 10 wherein the at least one of the interferon receptor binding domains comprises: amino acid residues 10-35 (IFN receptor recognition site I), 78-107 (IFN receptor recognition site II), 123-166 (IFN receptor recognition site III), or an IFN receptor binding portion thereof, is incorporated into the scaffold.

12. The interferon receptor binding peptide construct of claim 11 wherein the scaffold is C219 scFv encoded by the nucleic acid sequence provided in SEQ. ID NO. 18.

13. The interferon receptor binding peptide construct of claim 12 wherein the interferon receptor binding domain is inserted into scFv at one of the CDR sites, altering the native CDR fragment.

14. The interferon receptor binding peptide construct of claim 13, wherein the interferon receptor binding domain when comprising interferon receptor recognition site I or an IFN receptor binding portion thereof, is inserted at CDR3; interferon receptor recognition site II or IFN receptor binding portion thereof is inserted at CDR2; and interferon receptor recognition site III or IFN receptor binding portion thereof is inserted at CDR1, altering the CDR regions.

15. The interferon receptor binding peptide construct of claim 11 wherein the interferon receptor binding domain comprises the amino acid sequence selected from the sequences consisting of interferon amino acid residues: 29-35, 78-95, 123-140, 130-140 and 123-156.

16. The inteferon receptor binding peptide construct of claim 15 wherein the interferon receptor binding domain comprises the amino acid sequence selected from the sequences consisting of amino acid sequences: CYS-LEU-LYS-ASP-ARG-HIS-ASP (SEQ. ID NO. 1); ASP-GLU-SER-LEU-LEU-GLU-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 2); ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-TYR-ALA-ASN-VAL-VAL-HIS-GLN-ILE-ASN-HIS (SEQ. ID NO. 3); TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 4); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA (SEQ. ID NO. 5); TYR-PHE-GLN-ARG-ILE-THR-LEU-TYR (SEQ. ID NO. 6); GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP (SEQ. ID NO. 7), GLU-SER-LEU-LEU-ASP-LYS-PHE-TYR-THR-GLU-LEU-TYR-GLN-GLN-LEU-ASN-ASP-LEU(SEQ.ID.NO. 20), and LEU-TYR-LEU-THR-GLU-LYS-LYS-TYR-SER-PRO-CYS-ALA(SEQ. ID. NO. 21).

17. The interferon receptor binding peptide construct of claim 16 wherein tthe interferon receptor binding domain comprises the nucleic acid sequences selected from the group consisting of : SEQ. ID. NO. 1, SEQ. ID. NO. 20; and SEQ. ID. NO. 21.

18. The interferon receptor binding peptide construct of claim 14, wherein only one interferon receptor binding domain is inserted per CDR site.

19. The interferon receptor binding peptide construct of claim 13 or 14 wherein two interferon binding domains are inserted into the scFv, one at each CDR site.

20. The interferon receptor binding peptide construct of claim 13 wherein three interferon binding domains are inserted into the scFv, one at each CDR site

21. A interferon receptor binding peptide construct comprising the amino acid sequence of SEQ ID NO. 22 or 33.

22. A nucleic acid sequence encoding anyone of the peptide constructs of claims 1-21.

23. A nucleic acid molecule comprising the nucleic acid sequence of SEQ. ID. NO. 29 or 32.

24. An expression vector comprising the nucleic acid sequence encoding anyone of the peptide constructs of claims 1-21 or 23.

25. An expression vector plasmid pSJF2 comprising the nucleic acid sequence encoding anyone of the peptide constructs of claims 1-21 or 23.

26. A cell comprising the expression vector of claim 24.

27. A DNA molecule comprising: a single contiguous DNA sequence coding for a single chain recombinant hybrid immunoglobulin molecule comprising (1) one or more interferon receptor binding peptides incorporated into (2) an immunoglobulin scaffold.

28. A use of the peptide construct of claim 21 in the manufacture of a medicament as an interferon mimetic.

29. A pharmaceutical composition comprising one of the peptide constructs of anyone of claims 1-21 and a pharmaceutically acceptable carrier.

30. A method of producing a cytokine mimetic comprising:

(i) comparing the configuration of a cytokine receptor binding domain with that of an scFv molecule;

(ii) determining a suitable site for insertion of the binding domain in the scFv molecule to ensure maintenance of the domains in a configuration suitable for binding the cytokine receptor;

(iii) inserting the binding domain in the said identified site from step (ii) in a manner which enables the binding domain to bind the corresponding site of the receptor, altering the native scFv molecules; optionally

(iv) repeating steps (i) to (iii) for each cytokine receptor binding domain for cytokines that have more than one domain.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 32

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
CysLeuLysAspArgHisAsp
15

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
AspGluSerLeuLeuGluLysPheTyrThrGluLeuTyrGlnGlnLeu
151015
AsnAsp

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
AsnGluThrIleValGluAsnLeuLeuAlaAsnValTyrHisGlnIle
151015
AsnHis

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
TyrLeuThrGluLysLysTyrSerProCysAla
1510

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
TyrPheGlnArgIleThrLeuTyrLeuThrGluLysLysTyrSerPro
151015

CysAla

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
TyrPheGlnArgIleThrLeuTyr
15

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
GluLeuTyrGlnGlnLeuAsnAsp
15

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
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151015
LeuLeuAlaGlnMetArgArgIleSerProPheSerCysLeuLysAsp
202530
ArgHisAspPheGlyPheProGlnGluGluPheAspGlyAsnGlnPhe
354045
GlnLysAlaGlnAlaIleSerTyrLeuHisGluMetIleGlnGlnThr
505560
PheAsnLeuPheSerThrLysAspSerSerAlaAlaTrpAspGluSer
65707580
LeuLeuGluLysPheTyrThrGluLeuTyrGlnGlnLeuAsnAspLeu
859095
GluAlaCysTyrIleGlnGluValGlyValGluGluThrProLeuMet
100105110
AsnValAspSerIleLeuAlaValArgLysTyrPheGlnArgIleThr
115120125
LeuTyrLeuThrGluLysLysTyrSerProCysAlaTrpGluValVal
130135140
ArgAlaGluIleMetArgSerPheSerLeuSerThrAsnLeuGlnGlu
145150155160
ArgLeuArgArgLysGlu
165

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids

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(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
CysAspLeuProGlnThrHisSerLeuGlySerArgArgThrLeuMet
151015
LeuLeuAlaGlnMetArgArgIleSerLeuPheSerCysLeuLysAsp
202530
ArgHisAspPheGlyPheProGlnGluGluPheXaaGlyAsnGlnPhe
354045
GlnLysAlaGluThrIleProValLeuHisGluMetIleGlnGlnIle
505560
PheAsnLeuPheSerThrLysAspSerSerAlaAlaTrpAspGluThr
65707580
LeuLeuAspLysPheTyrThrGluLeuTyrGlnGlnLeuAsnAspLeu
859095
GluAlaCysTyrIleGlnGlyValGlyValThrGluThrProLeuMet
100105110
LysGluAspSerIleLeuAlaValArgLysTyrPheGlnArgIleThr
115120125
LeuTyrLeuThrGluLysLysTyrSerProCysAlaTrpGluValVal
130135140
ArgAlaGluIleMetArgSerPheSerLeuSerThrAsnLeuGlnGlu
145150155160
SerLeuArgSerLysGlu
165

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
GlnThrHisSerLeuGlySerArgArgThrLeuMetLeuLeuAlaGln
151015
MetArgArgIleSerLeuPheSerCysLeuLysAspArgHisAspPhe
202530
GlyPheProGlnGluGluPheGlyAsnGlnPheGlnLysAlaGluThr
354045
IleProValLeuHisGluMetIleGlnGlnIlePheAsnLeuPheSer
505560
ThrLysAspSerSerAlaAlaTrpAspGluThrLeuLeuAspLysPhe
65707580
TyrThrGluLeuTyrGlnGlnLeuAsnAspLeuGluAlaCysTyrIle
859095
GlnGlyValGlyValThrGluThrProLeuMetLysGluAspSerIle
100105110
LeuAlaValArgLysTyrPheGlnArgIleThrLeuTyrLeuThrGlu
115120125
LysLysTyrSerProCysAlaTrpGluValValArgAlaGluIleMet
130135140
ArgSerPheSerLeuSer
145150

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
GlnThrHisSerLeuGlySerArgArgThrLeuMetLeuLeuAlaGln
151015
MetArgArgIleSerLeuPheSerCysLeuLysAspArgHisAspPhe
202530
GlyPheProGlnGluGluPheGlyAsnGlnPheGlnLysAlaGluThr
354045
IleProValLeuHisGluMetIleGlnGlnIlePheAsnLeuPheSer
505560
ThrLysAspSerSerAlaAlaTrpAspGluThrLeuLeuAspLysPhe
65707580
TyrThrGluLeuTyrGlnGlnLeuAsnAspLeuGluAlaCysTyrIle
859095
GlnGlyValGlyValThrGluThrProLeuMetLysGluAspSerIle
100105110
LeuAlaValArgLysTyrPheGlnArgIleThrLeuTyrLeuThrGlu
115120125
LysLysTyrSerProCysAlaTrpGluValValArgAlaGluIleMet
130135140
ArgSerPheSerLeuSer
145150

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
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151015
MetArgArgIleSerLeuPheSerCysLeuLysAspArgHisAspPhe
202530
GlyPheProGlnGluGluPheGlyAsnGlnPheGlnLysAlaGluThr
354045
IleProValLeuHisGluMetIleGlnGlnIlePheAsnLeuPheSer
505560
ThrLysAspSerSerAlaAlaTrpAspGluThrLeuLeuAspLysPhe
65707580
TyrThrGluLeuTyrGlnGlnLeuAsnAspLeuGluAlaCysTyrIle
859095
GlnGlyValGlyValThrGluThrProLeuMetLysGluAspSerIle
100105110
LeuAlaValArgLysTyrPheGlnArgIleThrLeuTyrLeuThrGlu
115120125
LysLysTyrSerProCysAlaTrpGluValValArgAlaGluIleMet
130135140
ArgSerPheSerLeuSer
145150

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(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
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LeuLeuAlaGlnMetArgArgIleSerLeuSerSerCysLeuMetAsp
202530
ArgHisAspPheGlyPheProGlnGluGluPheGlyAsnGlnPheGln
354045
LysAlaGluThrIleProValLeuHisLeuMetIleGlnGlnIlePhe
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AsnLeuPheSerThrLysAspSerSerAlaAlaTrpAspGluThrLeu
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GluAspSerIleLeuAlaValArgLysTyrPheGlnArgIleThrLeu
115120125
TyrLeuThrGluLysLysTyrSerProCysAlaTrpGluValValArg
130135140
AlaGluIleMetArgSerPheSerLeuSerThrAsnLeuGlnGluSer
145150155160
LeuArgSerLysGlu
165

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
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354045
LysAlaGluThrIleProValLeuHisLeuMetIleGlnGlnIlePhe
505560
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859095
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115120125
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130135140
AlaGluIleMetArgSerPheSerLeuSerThrAsnLeuGlnGluSer
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165

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 162 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
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151015
MetSerArgIleSerProSerSerCysLeuMetAspArgHisAspPhe
202530
GlyPheProGlnGluGluPheAspGlyAsnGlnPheGlnLysAlaPro
354045
AlaIleSerValHisLeuGluLeuIleGlnGlnIlePheAsnLeuPhe
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130135140
MetArgSerPheSerLeuSerThrAsnLeuGlnGluArgLeuArgArg
145150155160
LysGlu

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
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151015
CysGlnLysLeuLeuTrpGlnLeuAsnGlyArgLeuGluTyrCysLeu
202530
LysAspArgMetAsnPheAspIleProGluGluGluLysGlnLeuGln
354045
GlnPheGlnLysGluAspAlaAlaLeuThrIleTyrGluMetLeuGln
505560
AsnIlePheAlaIlePheArgGlnAspSerSerSerThrGlyTrpAsn
65707580
GluThrIleValGluAsnLeuLeuAlaAsnValValHisGlnAsnHis
859095
LeuLysThrValLeuGluGluLysLeuGluLysGluAspPheThrPhe

100105110
IleGlyLysLeuMetSerSerLeuHisLeuLysArgTyrTyrGlyArg
115120125
IleLeuHisTyrLeuLysAlaLysGluTyrSerHisCysAlaTrpThr
130135140
IleValAlaValGluIleLeuArgAsnPheTyrLeuIleAsnArgLeu
145150155160
ThrGlyTyrLeuArgAsn
165

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 168 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
CysAspLeuProGlnThrHisAsnLeuArgAsnLysArgAlaLeuThr
151015
LeuLeuValGlnMetArgArgLeuSerProLeuSerCysLeuLysAsp
202530
ArgLysAspPheGlyPheProGlnGluLysValAspAlaGlnGlnIle
354045
GlnLysAlaGlnAlaIleProValLeuSerGluLeuThrGlnGlnIle
505560
LeuAsnIlePheThrSerLysAspSerSerAlaAlaTrpAsnAlaThr
65707580
LeuLeuAspSerPheCysAsnAspLeuHisGlnCysLeuAsnAspLeu
859095
GlnAlaCysLeuMetGlnGluValGlyValGlnGluProProLeuThr
100105110
GlnGluAspSerLeuLeuAlaValArgLysTyrPheHisArgIleThr
115120125
ValValLeuArgGluLysLysHisSerProCysAlaTrpGluValVal
130135140
ArgAlaGluValValValArgAlaLeuSerSerSerAlaAsnLeuLeu
145150155160
AlaArgLeuSerGluGluLysGlu
165

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 756 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18
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TGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAAAAGAACTACTTGACCTGGTACCAGCAG
AAACCAGGGCAGCCTCCTAAACTGTTGATTTACTGGGCATCCACTAGGGAATCTGGGGTCCCTGAT
CGCTTCACAGGCAGTGGCTCTGGAACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAAGAC
CTGGCAGTTTATTACTGTCAGAATGATTATAGTTATCCGCTCACGTTCGGTGCTGGGACCAAGCTG
GAGCCGGGCGGCGGCGGCTCGGGCGGCGGCGGCTCCGGAAAATCCGGAGGCGGCGGCGAGGTTCAG
CTGCAGCAGTCTGGGGCTGAGCTTGTGAGGCCAGGGGCCTCAGTCAAGTTGTCCTGCACAGCTTCT
GGCTTTAACATTAAAGACGACTTTATGCACTGGGTGAAGCAGAGGCCTGAACAGGGCCTGGAGTGG
ATTGGAAGGATTGATCCTGCGAATGATAATACTAAATATGCCCCGAAGTTCCAGGACAAGGCCACT

ATAATTGCAGACACATCCTCCAACACAGCTTACCTGCAGCTCAGCAGCCTGACATCTGAGGACACT
GCCGTCTATTACTGTGCTAGAAGAGAGGTTTATAGTTACTATAGTCCCCTCGATGTCTGGGGCGCA
GGGACCACGGTCACCGTCCCCTCAGGATCC

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
GluSerLeuLeuAspLysPheTyrThrGluLeuTyrGlnGlnLeuAsnAspLeu

(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
LeuTyrLeuThrGluLysLysTyrSerProCysAla

(2) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: final protein sequence IFNscFv
(B) TYPE:
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

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Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala Thr Val
Ala Gln Ala Val Phe Val Arg Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu
Thr Val Thr Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu
Leu Asp Pro Glu Glu Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp
Glu Val Val Lys Ala Gly Gly Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro
Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg
Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln
Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Ser Tyr Pro Leu Pro
Phe Ser Cys Leu Lys Asp Arg His Asp Thr Phe Gly Ala Gly Thr Lys Leu Glu
Pro Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Lys Ser Gly Gly Gly Gly
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val
Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asp Phe Met His Trp
Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala
Asn Asp Asn Thr Lys Tyr Ala Pro Lys Phe Gln Asp Lys Ala Thr Ile Ile Ala
Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp
Thr Ala Val Tyr Tyr Cys Ala Arg Arg Glu Val Tyr Ser Tyr Tyr Ser Pro Leu
Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Pro Ser Gly Ser Glu Gln Lys
Leu Ile Ser Glu Glu Asp Leu Asn His His His His His
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(2) INFORMATION FOR SEQ ID NO:23:

9/11

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
5'-GCAAGTCCAGTCAGAGTCT**AC**TAACCAGTGGAAATCAAAAG-3'

(2) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
5'-CTTTTGATTTCCACTGGTTA**GT**AGACTCTGACTGGACTTGC-3'

(2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
5'-GAATGATTATAGTTATCCG**TTA**ACGTTCGGTGCTGGGACC-3'

(2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
5'-GGTCCCAGCACCGAACGT**TAA**CGGATAACTATAATCATTC-3'

(2) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
5' TGCTTGAAAGATAGGCACGAC- 3'

(2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
5' -TTGTATTTGACCGAAAAAAAATATTCCCCGTGCGCG- 3'

(2) INFORMATION FOR SEQ ID NO:29:

10/11

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3,604 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
5'-
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ATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATT
ACGAATTCATGAAAAAAACCGCTATCGCGATCGCAGTTGCACTGGCTGGTTTCGCTACCGTTGCGCAGGCCGTCTT
CGTACGAGACATTGTGATGACACAGTCTCCATCCTCCCTGACTGTGACAGCAGGAGAGAAGGTCACTATGAGCTG
CAAGTCCAGTCAGAGTCTACTAGATCCGGAAGAATTGTATTTGACCGAAAAAAAATATTCCCCGTGCGCGTGGGAA
GTGGTGAAAGCGGGCGGCTTGACCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAACTGTTGATTTACTGGGCA
TCCACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGCTCTGGAACAGATTTCACTCTCACCATCAGCA
GTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAGAATGATTATAGTTATCCGTTGCCCTTTTCCTGCTTGAAA
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CAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTC
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GCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGA-3'

(2) INFORMATION FOR SEQ ID NO:30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
5'-CTGGCAGTTTATTACTGTCAG-3'

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: unknown
(ii) MOLECULE TYPE: DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
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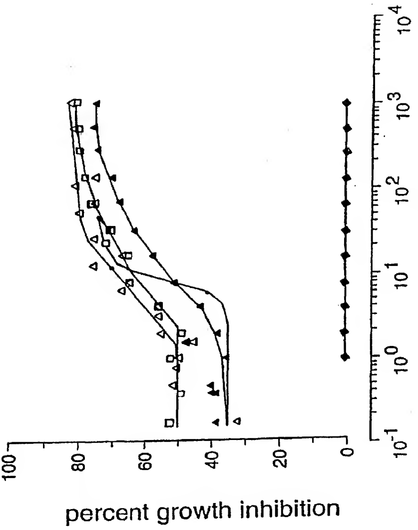

percent growth inhibition
100
80
60
40
20
0
ng / ml
10^-1
10^0
10^1
10^2
10^3
10^4

FIG.1.

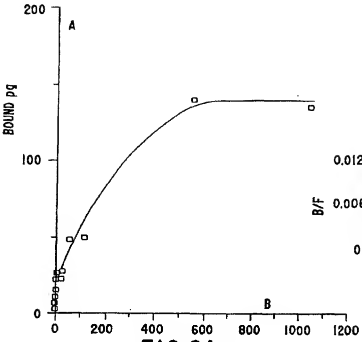


FIG.2A

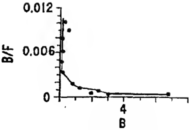


FIG.2A(a)

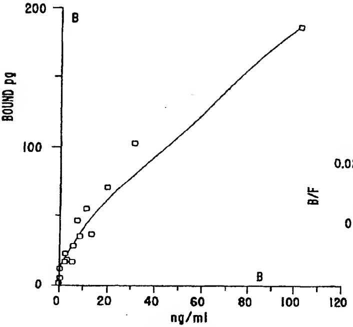


FIG.2B

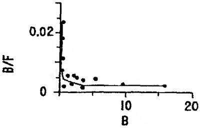


FIG.2B(b)

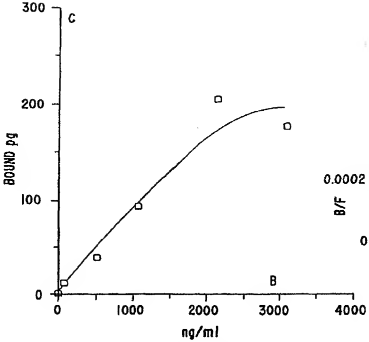


FIG.2C

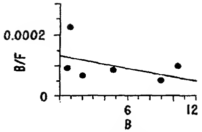


FIG.2C(c)

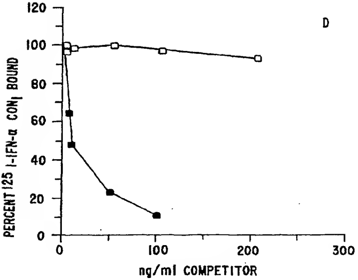


FIG.2D

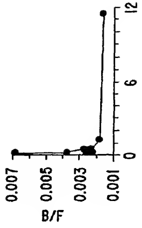


FIG.3A(a)

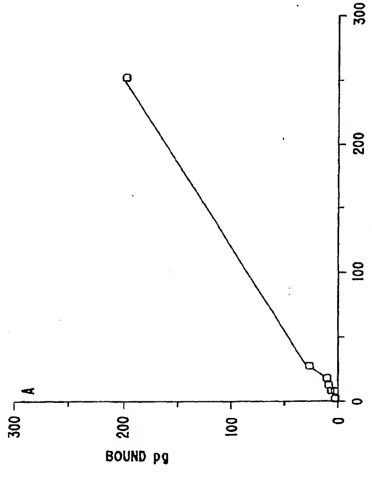


FIG.3A

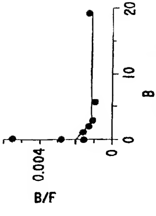


FIG.3B(b)

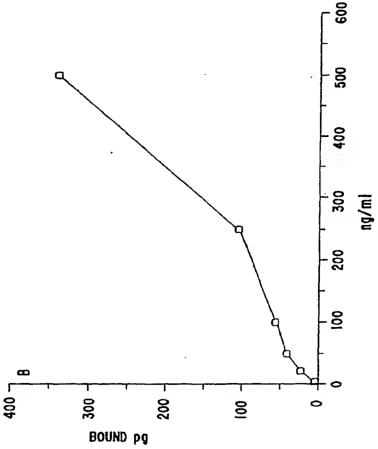


FIG.3B

FIG.4A(a)

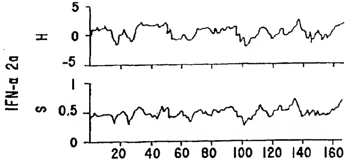


FIG.4A(b)

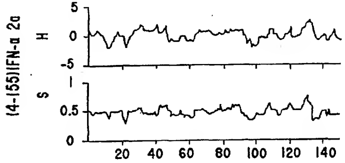


FIG.4A(c)

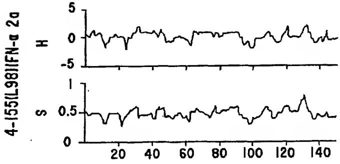


FIG.4A(d)

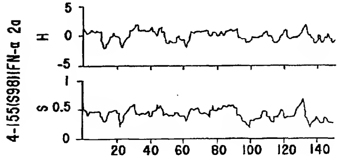


FIG.4B(a)

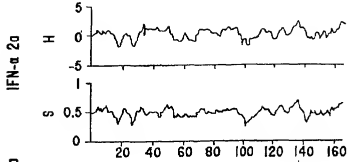


FIG.4B(b)

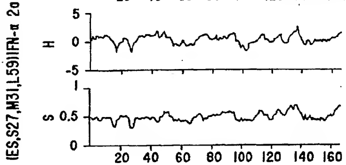


FIG.4B(c)

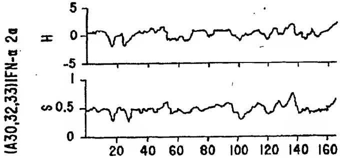


FIG.4B(d)

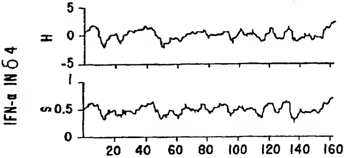


FIG.4C(a)

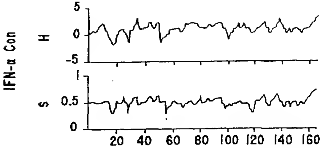


FIG.4C(b)

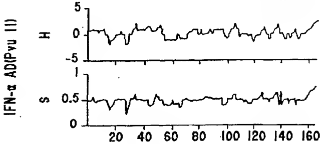


FIG.4C(c)

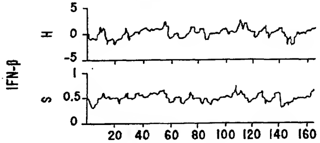


FIG.4C(d)

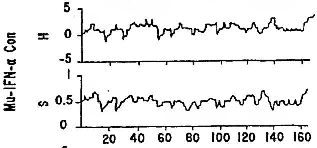
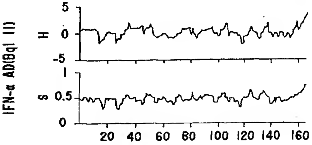


FIG.4C(e)



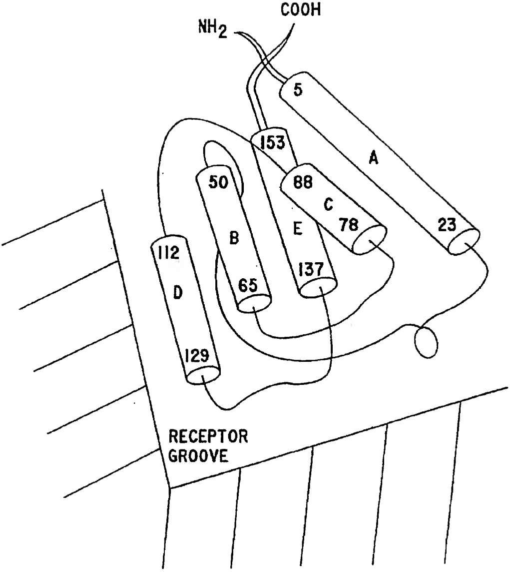


FIG.5

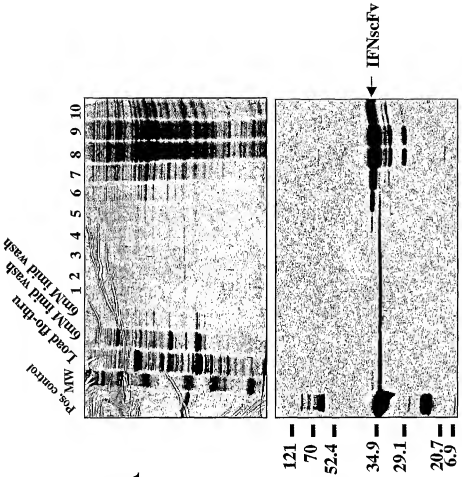

Pos. control
MW
Load flo-thru
6mM imid wash
6mM imid wash
1 2 3 4 5 6 7 8 9 10
IFNscFv
121
70
52.4
34.9
29.1
20.7
6.9

Fig. 6A

Fig. 6B

Fig. 7

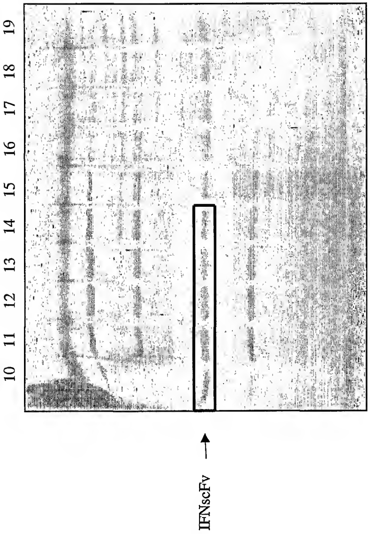


Fig. 8A

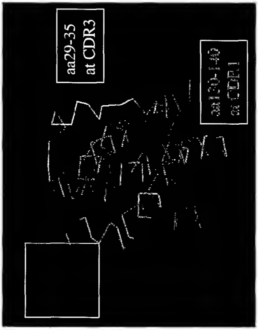
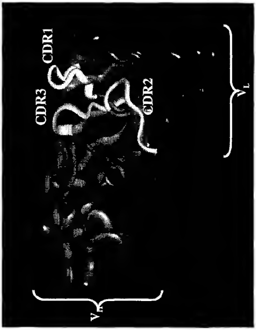


Fig. 8B



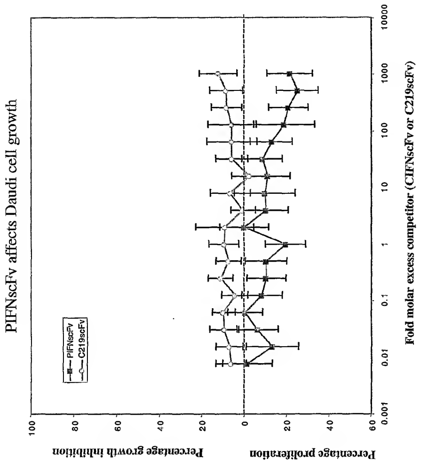

PIFNscFv affects Daudi cell growth
PIFNscFv
C219scFv
Percentage growth inhibition
Percentage proliferation
100
80
60
40
20
0
20
40
60
0.001
0.01
0.1
1
10
100
1000
10000
Fold molar excess competitor (CIFNscFv or C219scFv)

Fig. 9A

PIFNscFv can partially rescue Daudi cells from the growth inhibitory effects of IFN-Con

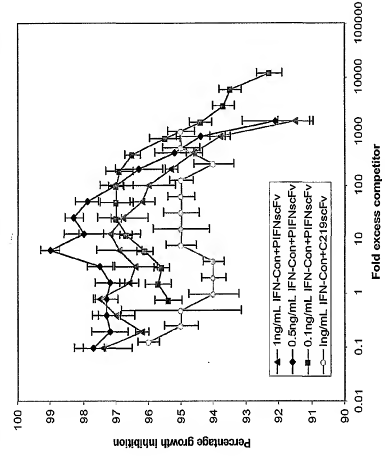
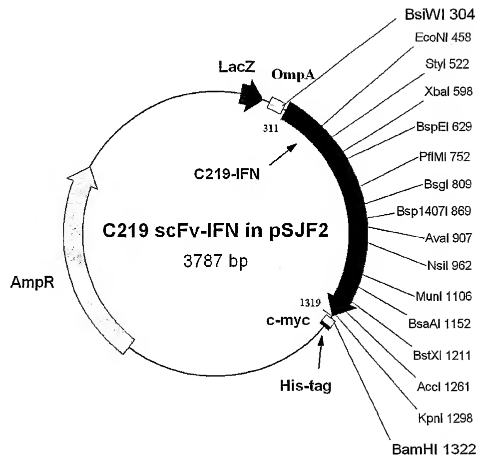


Fig. 9B

Panel C) Nucleotide sequences that were removed from the C219scFv cDNA and IFN nucleotide sequences that were placed in the CDRs.

C219scFv	IFN sequence
CDR2 of VL CTG TTG ATT TAC TGG GCA TCC ACT AGG GAA TCT GG GTC CCT GAT (SEQ.ID.NO.: 40)	**IRRP2** GGA GGC GGG TCT GTG CTT CAC GAA ATG ATT CAG CAG ACC TTT AAT TTA TTT TCG ACC AAG GAT TCG AGC GCT GCG TGG GAT GAG AGC CTG CTG GAG AAA TTT TAC ACG GAA CTC TAT CAG CTC AAT GAT CTA GAG GGT GGA GGT GGC (SEQ.ID.NO.: 34)
CDR1 of VH TTT AAC ATT AAA GAC GAC (SEQ.ID.NO.: 41)	**IRRP1** GGC GGC GGG AGC GGC GGC GGC GGC TCG GGC GGT GGA GGT TCT TGT CTG AAA GAT CGC CAT GAC TTC GGT GGC GGC (SEQ.ID.NO.: 35)
CDR3 of VH GTT TAT AGT TAC TAT AGT CCC CTC GAT (SEQ.ID.NO.: 42)	**IRRP3** GGC TAT TTC CAG CGT ATT ACC CTG TAC TTA ACC GAG AAA AAA TAC TCC CCA TGC GCG TGG GAA GTA GTG CGG GCA GAA ATC ATG CGT TCG TTC AGT TTG TCT ACA AAC CTG GGC GGA GGT GGA (SEQ.ID.NO.: 36)

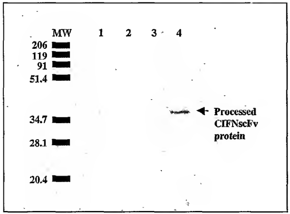
Fig. 10



Panel D) Plasmid map of the cIFNscFv construct in pSJF2 vector. The construct was directionally cloned from BsiWI to BamHI. The unique restriction sites introduced into the construct are indicated. Numbers beside the restriction sites refer to nucleotide numbers within the construct.

Fig. 11

Expression of CIFNscFv protein in TG1 *E.coli*



Anti-c-Myc Western blot
12% SDS-PAGE

20μL **whole cell lysates** from 100mL culture.
Induced for 3hrs at 30°C with 1mM IPTG.

Lane 1 TG1 cells containing empty pSJF2 vector prior to IPTG addition

Lane 2 TG1 cells containing empty pSJF2 vector post 3h IPTG induction

Lane 3 TG1 cells containing pSJF2-CIFNscFv prior to IPTG addition

Lane 4 TG1 cells containing pSJF2-CIFNscFv post 3h IPTG addition

Product is ~37kDa. Two bands are seen on the immunoblot. The higher band corresponds to unprocessed protein i.e., signal sequence is still present.

An anti-c-Myc Western immunoblot of whole cell lysates to screen for presence of the c-Myc tagged CIFNscFv mimetic. An IPTG inducible band, which corresponds to the expected molecular weight of the CIFNscFv mimetic, is present in lane 4

Fig. 12

18/28

FIG. 13

The following is the CIFNscFv cDNA construct sequence (SEQ. ID No. 32 is nucleic acid sequence, SEQ. ID No 33 is amino acid sequence)

```
1/1                                     31/11
ATG AAA AAA ACC GCT ATC GCG ATC GCA GTT GCA CTG GCT GGT TTC GCT ACC GTT GCG CAG
Met lys lys thr ala ile ala ile ala val ala leu ala gly phe ala thr val ala gln
61/21                                   91/31
GCC GTC TTC GTA CGa GAC ATC GTG ATG ACC CAA TCC CCA TCT TCG CTG ACA GTC ACG GCC
ala val phe val arg asp ile val met thr gln ser pro ser ser leu thr val thr ala
121/41                                  151/51
GGG GAA AAA GTG ACT ATG AGC TGT AAG AGC TCG CAG AGC TTA CTG AAT AGT GGT AAC CAG
gly glu lys val thr met ser cys lys ser ser gln ser leu leu asn ser gly asn gln
181/61                                  211/71
AAA AAT TAT CTG ACA TGG TAT CAG CAA AAA CCG GGT CAA CCG CCt AAA GGa GGC GGC GGG
lys asn tyr leu thr trp tyr gln gln lys pro gly gln pro pro lys gly gly gly gly
241/81                                  271/91
TCT GTG CTT CAC GAA ATG ATT CAG CAG ACC TTT AAT TTA TTT TCG ACc AAg GAt TCG AGC
ser val leu his glu met ile gln gln thr phe asn leu phe ser thr lys asp ser ser
301/101                                 331/111
GCT GCG TGG GAT GAG AGC CTG CTG GAG AAA TTT TAC ACG GAA CTC TAT CAG CAG CTC AAT
ala ala trp asp glu ser leu leu glu lys phe tyr thr glu leu tyr gln gln leu asn
361/121                                 391/131
GAT CTA GAG GGT GGA GGT GGC CGA TTC ACC GGG TCC GGa AGT GGC ACG GAT TTT ACT CTG
asp leu glu gly gly gly gly arg phe thr gly ser gly ser gly thr asp phe thr leu
421/141                                 451/151
ACC ATT AGC AGC GTT CAA GCG GAA GAT TTG GCG GTT TAT TAT TGT CAA AAT GAC TAC TCA
thr ile ser ser val gln ala glu asp leu ala val tyr tyr cys gln asn asp tyr ser
481/161                                 511/171
TAT CCG TTG ACT TTT GGA GCG GGT ACg AAA CTG GAA CCa GGC GGT GGG GGC TCT GGT GGT
tyr pro leu thr phe gly ala gly thr lys leu glu pro gly gly gly gly ser gly gly
541/181                                 571/191
GGG GGG TCG GGT AAG AGC GGA GGT GGT GGT GAA GTG CAG TTA CAG CAG AGT GGg GCt GAA
gly gly ser gly lys ser gly gly gly gly glu val gln leu gln gln ser gly ala glu
601/201                                 631/211
CTG GTC CGT CCA GGA GCC TCT GTT AAG CTG TCC TGt ACa GCA TCC GGT GGC GGC GGG AGC
leu val arg pro gly ala ser val lys leu ser cys thr ala ser gly gly gly gly ser
661/221                                 691/231
GGC GGC GGC GGC TCg GGC GGT GGA GGT TCT TGT CTG AAA GAT CGC CAT GAC TTC GGT GGC
gly gly gly gly ser gly gly gly gly ser cys leu lys asp arg his asp phe gly gly
721/241                                 751/251
GGC TTT ATG CAT TGG GTA AAA CAG CGC CCG GAA CAG GGG CTG GAA TGG ATC GGC CGC ATt
gly phe met his trp val lys gln arg pro glu gln gly leu glu trp ile gly arg ile
781/261                                 811/271
GAT CCT GCG AAC GAC AAC ACC AAA TAC GCC CCT AAA TTC CAA GAT AAG GCG ACC ATT ATT
asp pro ala asn asp asn thr lys tyr ala pro lys phe gln asp lys ala thr ile ile
841/281                                 871/291
GCT GAT ACC TCA AGC AAC ACT GCA TAT CTT CAA TTG TCA TCC CTT ACG AGT GAA GAC ACC
ala asp thr ser ser asn thr ala tyr leu gln leu ser ser leu thr ser glu asp thr
901/301                                 931/311
GCA GTT TAT TAC TGC GCa CGt aGG GAG GGC TAT TTC CAG CGT ATT ACC CTG TAC TTA ACC
ala val tyr tyr cys ala arg arg glu gly tyr phe gln arg ile thr leu tyr leu thr
961/321                                 991/331
GAG AAA AAA TAC TCC CCA TGC GCG TGG GAA GTA GTg CGg GCA GAA ATC ATG CGT TCG TTC
glu lys lys tyr ser pro cys ala trp glu val val arg ala glu ile met arg ser phe
1021/341                                1051/351
AGT tTg TCt ACA AAC CTG GGC GGA GGT GGA GTG TGG GGc GCc GGt ACC ACT GTG ACG GTC
ser leu ser thr asn leu gly gly gly gly val trp gly ala gly thr thr val thr val
1081/361                                1111/371
CCG TCA GGA TCC GAA CAA AAA CTG ATC AGC GAA GAA GAT CTG AAC CAT CAC CAT CAC CAT
pro ser gly ser glu gln lys leu ile ser glu glu asp leu asn his his his his his
1141/381
TAG TGA
AMB OPA
```

Fig. 14

IFN-α Primary Sequences

Molecular Weight
of aminopropylated IFN-α, excluding glycosylation

										10									20		
Con. Seq.	19710	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α1a	19748	C	D	L	P	E	T	H	S	L	D	N	R	R	T	L	M	L	L	A	Q
IFN-α1b	19776	C	D	L	P	E	T	H	S	L	D	N	R	R	T	L	M	L	L	A	Q
IFN-α2a	19554	C	D	L	P	Q	T	H	S	L	G	S	R	R	T	L	M	L	L	A	Q
IFN-α2b	19526	C	D	L	P	Q	T	H	S	L	G	S	R	R	T	L	M	L	L	A	Q
IFN-α2c	19573	C	D	L	P	Q	T	H	S	L	G	S	R	R	T	L	M	L	L	A	Q
IFN-α4a	19664	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α4b	19664	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α5	19731	C	D	L	P	Q	T	H	S	L	S	N	R	R	T	L	M	I	M	A	Q
IFN-α6	20031	C	D	L	P	Q	T	H	S	L	G	H	R	R	T	M	M	L	L	A	Q
IFN-α7a	19891	C	D	L	P	Q	T	H	S	L	R	N	R	R	A	L	I	L	L	A	Q
IFN-α7b	19978	C	D	L	P	Q	T	H	S	L	R	N	R	R	A	L	I	L	L	A	Q
IFN-α7c	19861	C	D	L	P	Q	T	H	S	L	R	N	R	R	A	L	I	L	L	A	Q
IFN-α8a	19779	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α8b	19769	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α8c	19168	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α10a	19691	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	G	Q
IFN-α10b	19804	C	D	L	P	Q	T	H	T	L	R	N	R	R	A	L	I	L	L	G	Q
IFN-α13	CODING SEQUENCE IDENTICAL TO IFN-α1																				
IFN-α14a	20027	C	N	L	S	Q	T	H	S	L	N	N	R	R	T	L	M	L	M	A	Q
IFN-α14b	19927	C	N	L	S	Q	T	H	S	L	N	N	R	R	T	L	M	L	M	A	Q
IFN-α14c	19993	C	N	L	S	Q	T	H	S	L	N	N	R	R	T	L	M	L	M	A	Q
IFN-α16	19567	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α17a	19545	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α17b	19585	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α17c	19628	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α17d	19638	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α21a	19585	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α21b	19567	C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q
IFN-α24	19901	C	N	L	S	Q	T	H	S	L	N	N	R	R	T	L	M	I	M	A	Q
IFN-ω		C	D	L	P	Q	N	H	G	L	L	S	R	N	T	L	V	L	L	H	Q
IFN-Con1		C	D	L	P	Q	T	H	S	L	G	N	R	R	A	L	I	L	L	A	Q

Fig. 14 (Cont'd)

									30									40		
Con. Seq.	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	G	F	P	Q
IFN-α1a	M	S	R	I	S	P	S	S	C	L	M	D	R	H	D	F	G	F	P	Q
IFN-α1b	M	S	R	I	S	P	S	S	C	L	M	D	R	H	D	F	G	F	P	Q
IFN-α2a	M	R	R	I	S	L	F	S	C	L	K	D	R	H	D	F	G	F	P	Q
IFN- α2b	M	R	K	I	S	L	F	S	C	L	K	D	R	H	D	F	G	F	P	Q
IFN- α2c	M	R	R	I	S	L	F	S	C	L	K	D	R	R	D	F	G	F	P	Q
IFN-α4a	M	G	R	I	S	H	F	S	C	L	K	D	R	H	D	F	G	F	P	E
IFN-α4β	M	G	R	I	S	H	F	S	C	L	K	D	R	H	D	F	G	F	P	E
IFN- α5	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	G	F	P	Q
IFN- α6	M	R	R	I	S	L	F	S	C	L	K	D	R	H	D	F	R	F	P	Q
IFN- α7a	M	G	R	I	S	P	F	S	C	L	K	D	R	H	E	F	R	F	P	E
IFN- α7b	M	G	R	I	S	P	F	S	C	L	K	D	R	H	E	F	R	F	P	E
IFN- α7c	M	G	R	I	S	P	F	S	C	L	K	D	R	H	E	F	R	F	P	E
IFN- α8a	M	R	R	I	S	P	F	S	C	L	K	D	R	H	D	F	E	F	P	Q
IFN- α8b	M	R	R	I	S	P	F	S	C	L	K	D	R	H	D	F	E	F	P	Q
IFN- α8c	M	R	R	I	S	P	F	S	C	L	K	D	R	H	D	F	E	F	P	Q
IFN- α10a	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	R	I	P	Q
IFN- α10b	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	R	I	P	Q
IFN- α13																				
IFN- α14a	M	R	R	I	S	P	F	S	C	L	K	D	R	H	D	F	E	F	P	Q
IFN- α14b	M	R	R	I	S	P	F	S	C	L	K	D	R	H	D	F	E	F	P	Q
IFN- α14c	M	R	R	I	S	P	F	S	C	L	K	D	R	H	D	F	E	F	P	Q
IFN- α16	M	G	R	I	S	H	F	S	C	L	K	D	R	Y	D	F	G	F	P	Q
IFN- α17a	M	G	R	I	S	P	F	S	C	L	K	D	R	P	D	F	G	L	P	Q
IFN- α17b	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	G	L	P	Q
IFN- α17c	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	G	L	P	Q
IFN- α17d	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	G	L	P	Q
IFN- α21a	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	G	F	P	Q
IFN- α21b	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	G	F	P	Q
IFN- α24	M	G	R	I	S	P	F	S	C	L	K	D	R	H	D	F	G	F	P	Q
IFN- ω	M	R	R	I	S	P	F	L	C	L	K	D	R	R	D	F	R	F	P	Q
IFN-Con1	M	R	R	I	S	P	F	S	C	L	K	D	R	H	D	F	G	F	P	Q

Fig. 14(Cont'd)

									50									60		
Con. Seq.	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN-α1a	E	E	F	D	G	N	Q	F	Q	K	A	P	A	I	S	V	L	H	E	L
IFN-α1b	E	E	F	D	G	N	Q	F	Q	K	A	P	A	I	S	V	L	H	E	L
IFN-α2a	E	E	F	-	G	N	Q	F	Q	K	A	E	T	I	P	V	L	H	E	M
IFN- α2b	E	E	F	-	G	N	Q	F	Q	K	A	E	T	I	P	V	L	H	E	M
IFN- α2c	E	E	F	-	G	N	Q	F	Q	K	A	E	T	I	P	V	L	H	E	M
IFN-α4a	E	E	F	D	G	H	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α4b	E	E	F	D	G	H	Q	F	Q	K	T	Q	A	I	S	V	L	H	E	M
IFN- α5	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α6	E	E	F	D	G	N	Q	F	Q	K	A	E	A	I	S	V	L	H	E	V
IFN- α7a	E	E	F	D	G	H	Q	F	Q	K	T	Q	A	I	S	V	L	H	E	M
IFN- α7b	E	E	F	D	G	H	Q	F	Q	K	T	Q	A	I	S	V	L	H	E	M
IFN- α7c	E	E	F	D	G	H	Q	F	Q	K	T	Q	A	I	S	V	L	H	E	M
IFN- α8a	E	E	F	D	D	K	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α8b	E	E	F	D	D	K	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α8c	E	E	F	D	D	K	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α10a	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α10b	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α13																				
IFN- α14a	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α14b	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α14c	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α16	E	V	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	A	F	H	E	M
IFN- α17a	E	E	F	D	G	N	Q	F	Q	K	T	Q	A	I	S	V	L	H	E	M
IFN- α17b	E	E	F	D	G	N	Q	F	Q	K	T	Q	A	I	S	V	L	H	E	M
IFN- α17c	E	E	F	D	G	N	Q	F	Q	K	T	Q	A	I	S	V	L	H	E	M
IFN- α17d	E	E	F	D	G	N	Q	F	Q	K	T	Q	A	I	P	V	L	H	E	M
IFN- α21a	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α21b	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- α24	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M
IFN- ω	E	M	V	K	G	S	Q	L	Q	K	A	H	V	M	S	V	L	H	E	M
IFN-Con1	E	E	F	D	G	N	Q	F	Q	K	A	Q	A	I	S	V	L	H	E	M

Fig. 14(Cont'd)

									70									80		
Con. Seq.	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	A	W	D	E	T
IFN-α1a	I	Q	Q	I	F	N	L	F	T	T	K	D	S	S	A	A	W	D	E	D
IFN-α1b	I	Q	Q	I	F	N	L	F	T	T	K	D	S	S	A	A	W	D	E	D
IFN-α2a	I	Q	Q	I	F	N	L	F	S	T	K	D	S	S	A	A	W	D	E	T
IFN- α2b	I	Q	Q	I	F	N	L	F	S	T	K	D	S	S	A	A	W	D	E	T
IFN- α2c	I	Q	Q	I	F	N	L	F	S	T	K	D	S	S	A	A	W	D	E	T
IFN-α4a	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α4b	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α5	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	T	W	D	E	T
IFN- α6	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	V	A	W	D	E	R
IFN- α7a	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α7b	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α7c	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α8a	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	A	L	D	E	T
IFN- α8b	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	A	L	D	E	T
IFN- α8c	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	A	L	D	E	T
IFN- α10a	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α10b	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α13																				
IFN- α14a	M	Q	Q	T	F	N	L	F	S	T	K	N	S	S	A	A	W	D	E	T
IFN- α14b	M	Q	Q	T	F	N	L	F	S	T	K	N	S	S	A	A	W	D	E	T
IFN- α14c	M	Q	Q	T	F	N	L	F	S	T	K	N	S	S	A	A	W	D	E	T
IFN- α16	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	A	W	D	E	T
IFN- α17a	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α17b	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α17c	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α17d	I	Q	Q	T	F	N	L	F	S	T	E	D	S	S	A	A	W	E	Q	S
IFN- α21a	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	A	W	E	Q	S
IFN- α21b	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	A	W	E	Q	S
IFN- α24	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	S	W	D	E	T
IFN- ω	L	Q	Q	I	F	S	L	F	H	T	E	R	S	S	A	A	W	N	M	T
IFN-Con1	I	Q	Q	T	F	N	L	F	S	T	K	D	S	S	A	A	W	D	E	S

Fig. 14(Cont'd)

									90									100		
Con. Seq.	L	L	D	K	F	Y	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN-α1a	L	L	D	K	F	C	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN-α1b	L	L	D	K	F	C	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN-α2a	L	L	D	K	F	Y	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α2b	L	L	D	K	F	Y	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α2c	L	L	D	K	F	Y	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN-α4a	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α4b	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α5	L	L	D	K	F	Y	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α6	L	L	D	K	L	Y	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α7a	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α7b	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α7c	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α8a	L	L	D	E	F	Y	I	E	L	D	Q	Q	L	N	D	L	E	V	L	C
IFN- α8b	L	L	D	E	F	Y	I	E	L	D	Q	Q	L	N	D	L	E	S	C	V
IFN- α8c	L	L	D	E	F	Y	I	E	L	D	Q	Q	L	N	D	L	E	V	L	C
IFN- α10a	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α10b	L	L	E	K	F	S	T	E	I	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α13																				
IFN- α14a	L	L	E	K	F	Y	I	E	L	F	Q	Q	M	N	D	L	E	A	C	V
IFN- α14b	L	L	E	K	F	Y	I	E	L	F	Q	Q	M	N	D	L	E	A	C	V
IFN- α14c	L	L	E	K	F	Y	I	E	L	F	Q	Q	M	N	D	L	E	A	C	V
IFN- α16	L	L	D	K	F	Y	I	E	L	F	Q	Q	L	N	D	L	E	A	C	V
IFN- α17a	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α17b	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α17c	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α17d	L	L	E	K	F	S	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V
IFN- α21a	L	L	E	K	F	S	T	E	L	N	Q	Q	L	N	D	M	E	A	C	V
IFN- α21b	L	L	E	K	F	S	T	E	L	N	Q	Q	L	N	D	L	E	A	C	V
IFN- α24	L	L	D	K	F	Y	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	M
IFN- ω	L	L	D	Q	L	H	T	G	L	H	Q	Q	L	Q	H	L	E	T	C	L
IFN-Con1	L	L	E	K	F	Y	T	E	L	Y	Q	Q	L	N	D	L	E	A	C	V

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Fig. 14(Cont'd)

	110 120
Con. Seq.	I Q E V G V E E T P L M N E D S I L A V
IFN-α1a	M Q E E R V G E T P L M N A D S I L A V
IFN-α1b	M Q E E R V G E T P L M N V D S I L A V
IFN-α2a	I Q G V G V T E T P L M K E D S I L A V
IFN- α2b	I Q G V G V T E T P L M K E D S I L A V
IFN- α2c	I Q G V G V T E T P L M K E D S I L A V
IFN-α4a	I Q E V G V E E T P L M N E D S I L A V
IFN- α4b	I Q E V G V E E T P L M N V D S I L A V
IFN- α5	M Q E V G V E D T P L M N V D I S L T V
IFN- α6	M Q E V W V G G T P L M N E D S I L A V
IFN- α7a	I Q E V G V E E T P L M N E D F I L A V
IFN- α7b	I Q E V G V E E T P L M N E D F I L A V
IFN- α7c	I Q E V G V E E T P L M N E D F I L A V
IFN- α8a	D Q E V G V I E S P L M Y E D S I L A V
IFN- α8b	M Q E V G V I E S P L M Y E D S I L A V
IFN- α8c	M Q E V G V I E S P L M Y E D S I L A V
IFN- α10a	I Q E V G V E E T P L M N E D S I L A V
IFN- α10b	I Q E V G V E E T P L M N E D S I L A V
IFN- α13	
IFN- α14a	I Q E V G V E E T P L M N E D S I L A V
IFN- α14b	I Q E V G V E E T P L M N E D S I L A V
IFN- α14c	I Q E V G V E E T P L M N E D S I L A V
IFN- α16	T Q E V G V E E I A L M N E D S I L A V
IFN- α17a	I Q E V G M E E T P L M N E D S I L A V
IFN- α17b	I Q E V G M E E T P L M N E D S I L A V
IFN- α17c	I Q E V G M E E T P L M N E D S I L A V
IFN- α17d	I Q E V G M E E T P L M N E D S I L A V
IFN- α21a	I Q E V G V E E T P L M N V D S I L A V
IFN- α21b	I Q E V G V E E T P L M N V D S I L A V
IFN- α24	M Q E V G V E D T P L M N V D S I L T V
IFN- ω	L Q V V G E G E S A G A I S S P A L T L
IFN-Con1	I Q E V G V E E T P L M N V D S I L A V

Fig. 14(Cont'd)

	130	140
Con. Seq.	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN-α1a	K K Y F R R I T L Y L T E K K Y S P C A	
IFN-α1b	K K Y F R R I T L Y L T E K K Y S P C A	
IFN-α2a	R K Y F Q R I T L Y L K E K K Y S P C A	
IFN- α2b	R K Y F Q R I T L Y L K E K K Y S P C A	
IFN- α2c	R K Y F Q R I T L Y L K E K K Y S P C A	
IFN-α4a	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α4b	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α5	R K Y T Q R I T L Y L T E K K Y S P C A	
IFN- α6	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α7a	R K Y F Q R I T L Y L M E K K Y S P C A	
IFN- α7b	R K Y F Q R I T L Y L M E K K Y S P C A	
IFN- α7c	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α8a	R K Y F Q R I T L Y L T E K K Y S S C A	
IFN- α8b	R K Y F Q R I T L Y L T E K K Y S S C A	
IFN- α8c	R K Y F Q R I T L Y L T E K K Y S S C A	
IFN- α10a	R K Y F Q R I T L Y L I E R K Y S P C A	
IFN- α10b	R K Y F Q R I T L Y L I E R K Y S P C A	
IFN- α13		
IFN- α14a	K K Y F Q R I T L Y L M E K K Y S P C A	
IFN- α14b	K K Y F Q R I T L Y L M E K K Y S P C A	
IFN- α14c	K K Y F Q R I T L Y L M E K K Y S P C A	
IFN- α16	R K Y F Q R I T L Y L M G K K Y S P C A	
IFN- α17a	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α17b	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α17c	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α17d	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α21a	K K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α21b	K K Y F Q R I T L Y L T E K K Y S P C A	
IFN- α24	R K Y F Q R I T L Y L T E K K Y S P C A	
IFN- ω	R R T F Q G I R V Y L K E K K Y S D C A	
IFN-Con1	K K Y F Q R I T L Y L T E K K Y S P C A	

Fig. 14(Cont'd)

										150									160		
Con. Seq.	W	E	V	V	R	A	E	I	M	R	S	F	S	F	S	T	N	L	Q	K
IFN-α1a	W	E	V	V	R	A	E	I	M	R	S	L	S	L	S	T	N	L	Q	E
IFN-α1b	W	E	V	V	R	A	E	I	M	R	S	L	S	L	S	T	N	L	Q	E
IFN-α2a	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	T	N	L	Q	E
IFN-α2b	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	T	N	L	Q	E
IFN-α2c	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	T	N	L	Q	E
IFN-α4a	W	E	V	V	R	A	E	I	M	R	S	L	S	F	S	T	N	L	Q	K
IFN-α4b	W	E	V	V	R	A	E	I	M	R	S	L	S	F	S	T	N	L	Q	K
IFN-α5	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	A	N	L	Q	E
IFN-α6	W	E	V	V	R	A	E	I	M	R	S	F	S	S	S	R	N	L	Q	E
IFN-α7a	W	E	V	V	R	A	E	I	M	R	S	F	S	F	S	T	N	L	K	K
IFN-α7b	W	E	V	V	R	A	E	I	M	R	S	F	S	F	S	T	N	L	Q	K
IFN-α7c	W	E	V	V	R	A	E	I	M	R	S	F	S	F	S	T	N	L	K	K
IFN-α8a	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	I	N	L	Q	K
IFN-α8b	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	I	N	L	Q	K
IFN-α8c	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	I	N	L	Q	K
IFN-α10a	W	E	V	V	R	A	E	I	M	R	S	L	S	F	S	T	N	L	Q	K
IFN-α10b	W	E	V	V	R	A	E	I	M	R	S	L	S	F	S	T	N	L	Q	K
IFN-α13																				
IFN-α14a	W	E	V	V	R	A	E	I	M	R	S	F	S	F	S	T	N	L	Q	K
IFN-α14b	W	E	V	V	R	A	E	I	M	R	S	F	S	F	S	T	N	L	K	K
IFN-α14c	W	E	V	V	R	A	E	I	M	R	S	L	S	F	S	T	N	L	Q	K
IFN-α16	W	E	V	V	R	A	E	I	M	R	S	F	S	F	S	T	N	L	Q	K
IFN-α17a	W	E	V	V	R	A	E	I	M	R	S	L	S	F	S	T	N	L	Q	K
IFN-α17b	W	E	V	V	R	A	E	I	M	R	S	L	S	F	S	T	N	L	Q	K
IFN-α17c	W	E	V	V	R	A	E	I	M	R	S	L	S	F	S	T	N	L	Q	K
IFN-α17d	W	E	V	V	R	A	E	I	M	R	S	L	S	F	S	T	N	L	Q	K
IFN-α21a	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	K	I	F	Q	E
IFN-α21b	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	K	I	F	Q	E
IFN-α24	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	K	I	F	Q	E
IFN-ω	W	E	V	V	R	M	E	I	M	K	S	L	F	L	S	T	N	M	Q	E
IFN-Con1	W	E	V	V	R	A	E	I	M	R	S	F	S	L	S	T	N	L	Q	E

Fig. 14(Cont'd)

	166
Con. Seq.	R L R R K E
IFN-α1a	R L R R K E
IFN-α1b	R L R R K E
IFN-α2a	S L R S K E
IFN- α2b	S L R S K E
IFN- α2c	S L R S K E
IFN-α4a	R L R R K D
IFN- α4b	R L R R K D
IFN- α5	R L R R K E
IFN- α6	R L R R K E
IFN- α7a	G L R R K D
IFN- α7b	R L R R K D
IFN- α7c	G L R R K D
IFN- α8a	R L K S K E
IFN- α8b	R L K S K E
IFN- α8c	D - - - - -
IFN- α10a	R L R R K D
IFN- α10b	R L R R K D
IFN- α13	
IFN- α14a	R L R R K D
IFN- α14b	G L R R K D
IFN- α14c	R L R R K D
IFN- α16	G L R R K D
IFN- α17a	I L R R K D
IFN- α17b	I L R R K D
IFN- α17c	R L R R K D
IFN- α17d	R L R R K D
IFN- α21a	R L R R K E
IFN- α21b	R L R R K E
IFN- α24	R L R R K E
IFN- ω	R L R S K D R D L G S S
IFN-Con1	R L R R K E

FIG. 15

The C219scFv sequence begins at amino acid postion 26 (Arg Ile Val, etc). The sequence 5' to the scFv protein codes for the OmpA sequence. aa278-288 is the c-Myc tag, the His tag follows.

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1/1                                     31/11
ATG AAA AAA ACC GCT ATC GCG ATC GCA GTT GCA CTG GCT GGT TTC GCT ACC GTT GCG CAG
Met lys lys thr ala ile ala ile ala val ala leu ala gly phe ala thr val ala gln

61/21                   SEQ. ID. NO. 18 -->  91/31
GCC GTC TTC GTA CGa|GAC ATT GTG ATG ACA CAG TCT CCA TCC TCC CTG ACT GTG ACA GCA
ala val phe val arg|asp ile val met thr gln ser pro ser ser leu thr val thr ala
                    SEQ. ID. NO. 19 -->
121/41                                  151/51
GGA GAG AAG GTC ACT ATG AGC TGC AAG TCC AGT CAG AGT CTG TTA AAC AGT GGA AAT CAA
gly glu lys val thr met ser cys lys ser ser gln ser leu leu asn ser gly asn gln

181/61                                  211/71
AAG AAC TAC TtG ACC TGG TAC CAG CAG AAA CCA GGG CAG CCT CCT AAA CTG TTG ATT TAC
lys asn tyr leu thr trp tyr gln gln lys pro gly gln pro pro lys leu leu ile tyr

241/81                                  271/91
TGG GCA TCC ACT AGG GAA TCT GGG GTC CCT GAT CGC TTC ACA GGC AGT GGC TCT GGA ACA
trp ala ser thr arg glu ser gly val pro asp arg phe thr gly ser gly ser gly thr

301/101                                 331/111
GAT TTC ACT CTC ACC ATC AGC AGT GTg cAG GCT GAA GAC CTG GCA GTT TAT TAC TGT CAG
asp phe thr leu thr ile ser ser val gln ala glu asp leu ala val tyr tyr cys gln

361/121                                 391/131
AAT GAT TAt agT TAT CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG Ccg ggc ggc ggc
asn asp tyr ser tyr pro leu thr phe gly ala gly thr lys leu glu pro gly gly gly

421/141                                 451/151
ggc tcg ggc ggc ggc ggc tcc GGA AAA TCC gga ggc ggc ggc GAG GTT CAG CTG CAG CAG
gly ser gly gly gly gly ser gly lys ser gly gly gly gly glu val gln leu gln gln

481/161                                 511/171
TCT GGG GCT GAG CTT GTG AGG CCA GGG GCC TCA GTC AAG TTG TCC TGC ACA GCT TCT GGC
ser gly ala glu leu val arg pro gly ala ser val lys leu ser cys thr ala ser gly

541/181                                 571/191
TTT AAC ATT AAA GAC GAC TTT ATG CAC TGG GTG AAG CAG AGG CCT GAA CAG GGC CTG GAG
phe asn ile lys asp asp phe met his trp val lys gln arg pro glu gln gly leu glu

601/201                                 631/211
TGG ATT GGA AGG ATT GAT CCT GCG AAT GAT AAT ACT AAA TAT GCC CCG AAG TTC CAG GAC
trp ile gly arg ile asp pro ala asn asp asn thr lys tyr ala pro lys phe gln asp

661/221                                 691/231
AAG GCC ACT ATA AtT GCA GAC ACA TCC TCC AAC ACA GCt TAC CTG CAG CTC AGC AGC CTG
lys ala thr ile ile ala asp thr ser ser asn thr ala tyr leu gln leu ser ser leu

721/241                                 751/251
ACA TCT GAG GAC ACT GCC GTC TAT TAC TGT GCT AGA AGA GAG GTT TAT AGT TAC TAT AGT
thr ser glu asp thr ala val tyr tyr cys ala arg arg glu val tyr ser tyr tyr ser

781/261                                 811/271
CCC CTC GAT GTC TGG GGC GCA GGG ACC ACG GTC ACC GTC cCC TCA|GGA TCC GAA CAA AAA
pro leu asp val trp gly ala gly thr thr val thr val pro ser|gly ser glu gln lys

841/281                                 871/291
CTG ATC AGC GAA GAA GAT CTG AAC CAT CAC CAT CAC CAT TAG TGA
leu ile ser glu glu asp leu asn his his his his his AMB OPA
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